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(54) Title: THERAPEUTIC MOLECULES AND METHODS-1

(57) Abstract: Methods of inhibiting the cytokine or biological activity of Macrophage Migration Inhibitory Factor (MIF) compris-
ing contacting MIF with a compound of formula (I) as defined herein, is provided. The invention also relates to methods of treating
diseases or conditions where MIF cytokine or biological activity is implicated comprising administration of compounds of formula
(I), either alone or as part of a combination therapy. Novel heterocyclic compounds are also provided for.

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THERAPEUTIC MOLECULES AND METHODS – 1

FIELD OF THE INVENTION

5 The present invention relates generally to the treatment of diseases or conditions resulting from cellular activation, such as inflammatory or cancerous diseases or conditions. In particular, the invention relates to the use of heterocyclic derivatives to inhibit the cytokine or biological activity of macrophage migration inhibitory factor (MIF), and diseases or conditions wherein MIF cytokine or biological activity is implicated.

10

BACKGROUND OF THE INVENTION

MIF is the first identified T-cell-derived soluble lymphokine. MIF was first described as a soluble factor with the ability to modify the migration of macrophages (1). The molecule responsible for the biological actions ascribed to MIF was identified and cloned in 1989 (2). Initially found to activate macrophages at inflammatory sites, it has been shown to possess pluripotential actions in the immune system. MIF has been shown to be expressed in human diseases which include inflammation, injury, ischaemia or malignancy. MIF also has a unique relationship with glucocorticoids by overriding their anti-inflammatory effects.

20

Recent studies have indicated that monoclonal antibody antagonism of MIF may be useful in the treatment of sepsis, certain types of cancers and delayed type hypersensitivity. Antibody antagonism of MIF has also been shown to have activity in adjuvant- or collagen-induced arthritis animal models and other models of inflammatory and immune diseases.

25

Although antibody antagonism of MIF is one potential way to provide therapeutic treatments, such biological molecules can be expensive to prepare on a commercial basis and further, can be limited in the way they are administered (generally by injection) and do not readily lend themselves to formulations for administration by other means eg oral

30

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administration.

Small molecule inhibitors may overcome one or more such difficulties connected with the use of biological therapeutic treatments. There exists a need, therefore, for small molecule inhibitors of the cytokine or biological activity of MIF. Small molecule inhibitors of the MIF would have therapeutic effects in a broad range of diseases, whether given alone or in combination with other therapies.

Examples of agents which could be used in combination with a compound of formula (I) include glucocorticoids, antirheumatic drugs, immunosuppressive drugs, anti-cytokine therapies, antagonists or inhibitors of mitogen-activated protein (MAP) kinases, antagonists or inhibitors of nuclear factor kappa-B (NF- κ B) signal transduction pathway, antibodies, protein therapeutics or small molecule therapeutics interacting with adhesion molecules and co-stimulatory molecules, bronchodilators, antagonists of eicosanoid synthesis pathways, agents used for the treatment of inflammatory bowel disease, anti-cancer drugs, antisense oligonucleotides, interfering RNA and ribozymes.

For example, glucocorticoids have been used to treat human diseases for over fifty years and are effective in a range of diseases which include inflammation, injury, ischaemia or malignancy. Although debate continues in relation to their impact on disease prognosis, their influence on symptoms and signs of inflammation, especially in the short term, can be dramatic.

Despite their benefits and efficacy, the use of glucocorticoids is limited by universal, predictable, dose-dependent toxicity. Mimicking Cushing's disease, a disease wherein the adrenal glands produce excess endogenous glucocorticoids, glucocorticoid treatment is associated with side effects including immunosuppression (resulting in increased susceptibility to infections), weight gain, change in body habitus, hypertension, oedema, diabetes mellitus, cataracts, osteoporosis, poor wound healing, thinning of the skin, vascular fragility, hirsutism and other features of masculinization (in females). In children, growth retardation is also noted. These side effects are known as Cushingoid side effects.

- Since the side effects of glucocorticoids are dose dependent, attempts to reduce the dosage requirement have been investigated, including combination therapies in which glucocorticoids are administered with other therapeutic agents. These combination therapies are sometimes referred to as "steroid-sparing" therapies. However, currently available combination therapies are non-specific as the other therapeutic agents do not address biological events which inhibit the effectiveness of glucocorticoids. Such combination therapies are also typically associated with serious side effects.
- 10 Furthermore, glucocorticoids are incompletely effective in a number of disease settings, leading to the concept of "steroid-resistant" diseases. Agents which amplify or enhance the effects of glucocorticoids would not only allow the reduction of dose of these agents but may also potentially render "steroid-resistant" diseases steroid-sensitive.
- 15 There is a need for effective therapies which enable a reduction in the dosage level of glucocorticoids. There is also a need for effective treatment of "steroid-resistant" diseases. Preferably, such therapies or treatments would address factors which directly limit the effectiveness of glucocorticoids.
- 20 Therapeutic antagonism of MIF may provide "steroid-sparing" effects or be therapeutic in "steroid-resistant" diseases. Unlike other pro-inflammatory molecules, such as cytokines, the expression and/or release of MIF can be induced by glucocorticoids (3), (4). Moreover, MIF is able to directly antagonize the effects of glucocorticoids. This has been shown to be the case for macrophage TNF, IL-1 β , IL-6 and IL-8 secretion (5), (6), and for
- 25 T cell proliferation and IL-2 release (7). *In vivo*, MIF exerts a powerful glucocorticoid-antagonist effect in models including endotoxic shock and experimental arthritis (5), (8). In the context of an inflammatory or other disease treated with glucocorticoids, then, MIF is expressed but exerts an effect which prevents the glucocorticoid inhibition of inflammation. It can therefore be proposed that therapeutic antagonism of MIF would
- 30 remove MIF's role in inhibiting the anti-inflammatory effect of glucocorticoids, thereby allowing glucocorticoids to prevail. This would be the first example of true "steroid-

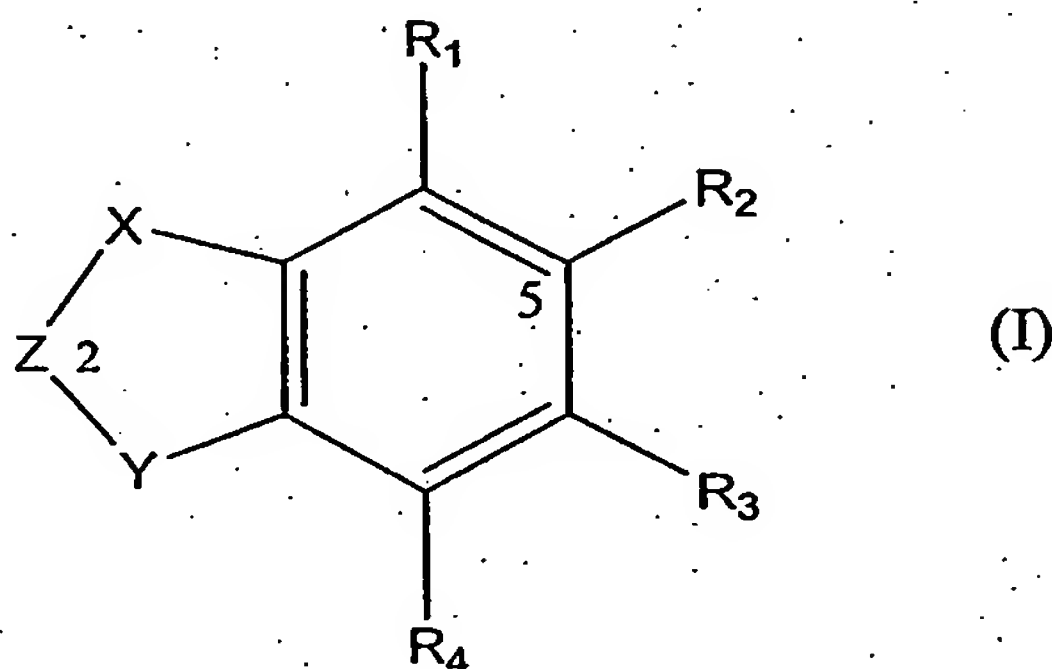
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sparing" therapy. In support of this hypothesis is the observation that anti-MIF antibody therapy reverses the effect of adrenalectomy in rat adjuvant arthritis (9). By neutralizing the natural glucocorticoid 'counter-regulator' effect of MIF, it is envisioned that with MIF antagonism, steroid dosages could be reduced or even eliminated in inflammatory disease, particularly in those diseases that are associated with the glucocorticoid resistance (10), (11). There is a need, therefore, for therapeutic antagonists of the cytokine or biological activity of MIF.

10 SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

In a first aspect, the present invention provides a method of inhibiting cytokine or biological activity of MIF comprising contacting MIF with a cytokine or biological activity inhibiting effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof



wherein

25 X is selected from -O-, -S-, -C(R₅)(R₅)- or -N(R₆)- and preferably comprises a hydrogen bond donor or acceptor;

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Y is selected from -N(R₇)-, -O-, -S- or -C(R₇)₂-;

Z is selected from -C(O)-, -C(S)-, -C(=NR₆)-, -S(O)- or -S(O)₂-;

5

R₁ is selected from hydrogen, C₁₋₃alkyl, (CR₅R_{5'})_nOR₇, (CR₅R_{5'})_nSR₇, (CR₅R_{5'})_nN(R₆)₂ and (CR₅R_{5'})_nhalo;

10

R₂ is selected from C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R_{12'})_mC(O)R₈, (CR₁₂R_{12'})_mC(S)R₈, (CR₁₂R_{12'})_mS(O)R₈, (CR₁₂R_{12'})_mS(O)₂R₈, (CR₁₂R_{12'})_mOR₉, (CR₁₂R_{12'})_mSR₉, (CR₁₂R_{12'})_mNR₁₀R₁₁, (CR₁₂R_{12'})_mC(=NR₂₄)R₂₂ and (CR₁₂R_{12'})_mR₁₃;

15

R₃ is selected from hydrogen, C₁-C₆alkyl, (CR₁₆R_{16'})_pNR₁₄R₁₅, (CR₁₆R_{16'})_pOR₁₇, (CR₁₆R_{16'})_pSR₁₇, (CR₁₆R_{16'})_phalo, (CR₁₆R_{16'})_pNO₂, (CR₁₆R_{16'})_nC(O)R₂₈, (CR₁₆R_{16'})_nC(=NR₂₄)R₂₂, (CR₁₆R_{16'})_nS(O)R₁₇, (CR₁₆R_{16'})_nS(O)₂R₁₇, (CR₁₆R_{16'})_nS(O)₃R₁₇ and (CR₁₆R_{16'})_pC(R₁₈)₃;

20

R₄ is selected from hydrogen, halogen C₁-C₃alkyl, C₂₋₃alkenyl, C₂₋₃alkynyl and (CR₁₂R_{12'})_nC(R₁₈)₃;

Each R₅ and R_{5'} is independently selected from hydrogen, C₁-C₃alkyl, halo, OR₇, SR₇ and N(R₆)₂;

Each R₆ is independently selected from hydrogen, C₁-C₃alkyl and OR₇;

25

Each R₇ is independently selected from hydrogen and C₁-C₃alkyl;

R₈ is selected from hydrogen, C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, OR₁₉, SR₁₉, N(R₂₀)₂, [NH-CH(R₂₁)-C(O)]_q-OR₂₉, [sugar]_q and (CR₁₂R_{12'})_tR₁₃;

30

R₉ is selected from hydrogen, C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R_{12'})_tR₁₃,

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C(O)R₂₃, CO₂R₂₃, C(S)R₂₃, C(S)OR₂₃, S(O)R₂₃, S(O)₂R₂₃, [C(O)CH(R₂₁)NH]_q-R₂₃ and [sugar]_q;

R₁₀ and R₁₁ are independently selected from hydrogen, C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R_{12'})_mR₁₃, C(O)R₂₃, C(S)R₂₃, S(O)R₂₃, S(O)₂R₂₃, [C(O)CH(R₂₁)NH]_q-R₂₃, -[sugar]_q and NHC(=NR₂₅)-NH₂;

Each R₁₂ and R_{12'} is independently selected from hydrogen, C₁-C₆alkyl, C₂-C₆alkenyl, C₂-C₆alkynyl, OR₂₄, SR₂₄, halo, N(R₂₄)₂, CO₂R₂₄, CN, NO₂, aryl or heterocyclyl;

R₁₃ is selected from OR₂₅, SR₂₅, halo, N(R₂₅)₂, C(O)R₃₁, CN, C(R₁₈)₃, aryl or heterocyclyl;

R₁₄ and R₁₅ are independently selected from hydrogen, C₁-C₃alkyl, OR₁₇, (CR₁₆R_{16'})_pC(R₁₈)₃;

Each R₁₆ and R_{16'} is independently selected from hydrogen, C₁-C₃alkyl, halo, OR₁₇, SR₁₇ and N(R₁₇)₂;

Each R₁₇ is independently selected from hydrogen and C₁-C₃alkyl;

Each R₁₈ is independently selected from hydrogen and halo;

R₁₉ and each R₂₀ are independently selected from hydrogen, C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₂₆R_{26'})_tR₂₇;

R₂₁ is the characterising group of an amino acid;

R₂₂ is selected from C₁-C₆alkyl, NH₂, NH(C₁₋₆alkyl), N(C₁₋₆alkyl)₂, OR₂₉ or SR₂₉;

R₂₃ is selected from hydrogen, C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, aryl (CR₂₆R_{26'})_tR₂₇;

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Each R_{24} is independently selected from hydrogen and C_1 - C_6 alkyl;

Each R_{25} is independently selected from hydrogen, C_1 - C_6 alkyl, C_{1-3} alkoxy C_{1-3} alkyl, aryl
5 and heterocyclyl;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl,
 C_2 - C_6 alkynyl, OR_{29} , SR_{29} , halo, $N(R_{29})_2$, CO_2R_{29} , CN, NO_2 , aryl and heterocyclyl;

10 R_{27} is selected from hydrogen, OR_{30} , SR_{30} , halo, $N(R_{30})_2$, CO_2R_{30} , aryl and heterocyclyl;

R_{28} is selected from hydrogen, C_{1-6} alkyl, OR_{29} , SR_{29} or $N(R_{29})_2$;

Each R_{29} is independently selected from hydrogen and C_1 - C_3 alkyl;

15

Each R_{30} is independently selected from hydrogen, C_1 - C_3 alkyl, aryl and heterocyclyl;

R_{31} is selected from C_{1-3} alkyl, OH, C_{1-3} alkoxy, aryl, aryloxy, heterocyclyl and
heterocyclyloxy;

20

n is 0 or an integer from 1 to 3;

m is 0 or an integer from 1 to 20;

p is 0 or an integer from 1 to 6;

q is an integer from 1 to 5;

25 t is an integer from 1 to 10;

wherein alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

In another aspect, the invention provides a method of treating, preventing or diagnosing a
30 disease or condition wherein MIF cytokine or biological activity is implicated comprising
the administration of a treatment, prevention or diagnostic effective amount of a compound

of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In a further aspect, there is provided the use of a compound of formula (I) or a
5 pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment, prevention or diagnosis of a disease or condition wherein MIF cytokine or biological activity is implicated.

In particular, the invention provides a method of treating, diagnosing or preventing
10 autoimmune diseases, solid or haemopoietic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis,
psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but
15 not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but
20 not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis),
25 pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer,
30 leukemia, cervical cancer, multiple myeloma and metastatic cancer),

atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg dementia, Alzheimer's disease, multiple sclerosis, demyelinating diseases), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, parturition, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing,

comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

A further aspect of the invention provides for the use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment of a disease or condition as above.

A further aspect of the invention provides a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof and a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the invention provides a method of treating or preventing a disease or condition wherein MIF cytokine or biological activity is implicated comprising:

administering to a mammal a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof and a second therapeutic agent.

- 10 -

In another aspect, the present invention provides a method of prophylaxis or treatment of a disease or condition for which treatment with a glucocorticoid is indicated, said method comprising:

- 5 administering to a mammal a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

In yet another aspect, the present invention provides a method of treating steroid-resistant diseases comprising:

- 10 administering to a mammal a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

- 15 In a further aspect, the present invention provides a method of enhancing the effect of a glucocorticoid in mammals comprising administering a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof, simultaneously, separately or sequentially with said glucocorticoid.

- 20 In yet a further aspect, the present invention provides a pharmaceutical composition comprising a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

- 25 In a further aspect of the invention there is provided a use of a glucocorticoid in the manufacture of a medicament for administration with a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

- 30 In yet a further aspect of the invention there is provided a use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for administration with a glucocorticoid for the treatment or prophylaxis of a disease or condition for which treatment of a glucocorticoid is indicated.

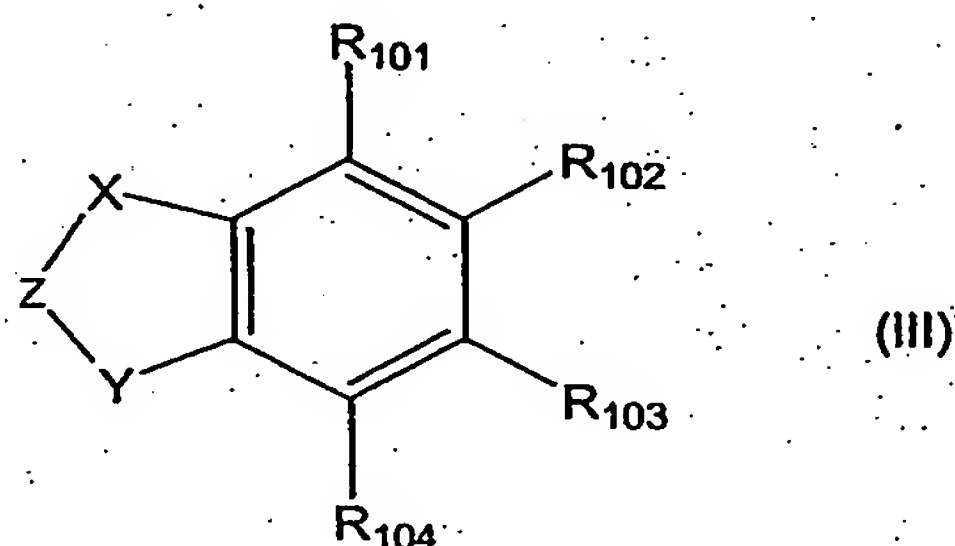
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In yet a further aspect of the invention there is provided a use of a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

In preferred embodiments, the compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof are used to treat or prevent a disease or condition, particularly in a human subject.

10

In yet a further aspect of the invention, there is provided a compound of formula (III) or a pharmaceutically acceptable salt or prodrug thereof:



15

wherein

X is -O-, -NH-, -O- or -CH₂-;

20 Y is -NH-, -O- or -CH₂-;

Z is -C(O)-, -C(S)- or -S(O)-;

R₁₀₁ is selected from hydrogen, C₁₋₃alkyl, OH, SH, NH₂, NHC₁₋₃alkyl, F, Cl or Br;

25

R₁₀₂ is selected from C₁₋₂₀alkyl, C₂₋₂₀alkenyl, CO₂H, CO₂R₁₀₅, -NH₂, F, Cl, Br, (CH₂)_wR₁₀₆, C(O)N(R₁₀₇)₂, C(=N)NHC₁₋₆alkyl, SO₂C₁₋₆alkyl, C(O)[NHCH(R₁₀₈)C(O)]_q-OR₁₀₉,

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C(O)sugar, C(O)NH(CH₂)_naryl, NHC(O)(CH₂)_nSheterocyclyl, C(O)SC₁₋₆alkyl,
C(O)(CH₂)_nCO₂H, SO₂OC₁₋₁₀alkyl and SO₂NHC₁₋₁₀alkyl;

R₁₀₃ is selected from hydrogen, F, Cl, Br, C₁₋₆alkyl, -(CH₂)_nNH₂, -(CH₂)_n-OH, -(CH₂)_n-
5 CF₃, -(CH₂)_nC(O)C₁₋₃alkyl or -(CH₂)_n-SH;

R₁₀₄ is selected from hydrogen, methyl, ethyl, CH₂C(R₁₁₀)₃, C(R₁₁₀)₃, -CH₂=CH₂, fluoro,
chloro or bromo;

10 R₁₀₅ is selected from hydrogen, C₁₋₂₀alkyl, C₂₋₂₀alkenyl or (CH₂)_tOC₁₋₃alkyl;

R₁₀₆ is selected from SH, SC₁₋₆alkyl, OH, OC₁₋₆alkyl, sugar, CO₂H, NH₂, heterocyclyl or
aryl;

15 Each R₁₀₇ is independently selected from hydrogen, C₁₋₂₀alkyl, C₂₋₂₀alkenyl, (CH₂)_taryl and
(CH₂)_theterocyclyl;

R₁₀₈ is the characterising group of an amino acid;

20 R₁₀₉ is hydrogen, C₁₋₃alkyl;

Each R₁₁₀ is independently selected from hydrogen and halo; and

n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6; t is
25 an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be
optionally substituted.

BRIEF DESCRIPTION OF THE FIGURES

30 Figure 1 graphically depicts dose response effect of benzimidazol-2-one-5-
pentanoate (compound 5) on Interleukin-1 (IL-1)-induced cyclooxygenase

II (COX-2) expression.

- Figure 2 graphically depicts the effect of a combination of dexamethasone and benzimidazol-2-one-5-pentanoate (compound 5) on IL-1 induced COX-2 expression.
- Figure 3 graphically depicts the effect of MIF antagonist, benzimidazol-2-one-5-pentanoate (compound 5) and dexamethasone on IL-1 induced phosphorylation (activation) of ERK (extracellular signal regulated kinase), as detected by Western blotting.
- Figure 4 graphically depicts the effect of benzimidazol-2-one-5-pentanoate (compound 5) on *in vivo* serum IL-6 production in a murine endotoxic shock model.
- Figure 5 graphically depicts the effect of benzimidazol-2-one-5-pentanoate (compound 5) on *in vivo* serum IL-1 production in a murine endotoxic shock model.
- Figure 6 graphically depicts the cytotoxicity effect of benzimidazol-2-one-5-pentanoate (compound 5) *in vitro*.
- Figure 7 graphically depicts the cytotoxicity effect of a number of compounds of formula (I) *in vitro*.
- Figure 8 depicts graphically the effect of benzimidazol-2-one-5 pentanoate (compound 5) on antigen-specific activation of splenic T lymphocytes from mice pre-immunised against mBSA. Activation is measured using tritiated (³H)-thymidine incorporation, as a measure of antigen-induced T cell proliferation.

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Figure 9 depicts graphically the effect of benzimidazol-2-one-5 pentanoate (compound 5) on cutaneous delayed-type hypersensitivity reactions in vivo.

Figure 10 depicts graphically the in vivo effects of benzimidazol-2-one-5 pentanoate (compound 5) on murine antigen induced arthritis, an animal model of rheumatoid arthritis.

Figure 11 depicts graphically the effect of in vivo treatment with compounds 5 and 13 on the ex vivo antigen-specific activation of lymph node and splenic T cells in response to mBSA.

Figure 12 depicts graphically the effect of benzimidazol-2-one-5 pentanoate (compound 5) on proliferation of cells treated with recombinant human MIF.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "alkyl", either used alone or in compound terms such as NHCalkyl, N(Calkyl)₂, etc., refers to monovalent straight, branched or, where appropriate, cyclic aliphatic radicals, having 1 to 3, 1 to 6, 1 to 10 or 1 to 20 carbon atoms as appropriate, e.g. methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, sec-butyl, t-butyl and cyclobutyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, cyclopentyl, n-hexyl, 1- 2- 3- or 4- methylpentyl, 1- 2- or 3-ethylbutyl, 1 or 2- propylpropyl or cyclohexyl.

An alkyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO₂, CO₂H, CO₂C₁₋₆alkyl, CONH₂, CONH(C₁₋₆alkyl), CONH(C₁₋₆alkyl)₂, OH, hydroxyalkyl, alkoxy, methyl, ethyl, propyl, butyl, methoxy, ethoxy, butyloxy, acyl, carboxyalkyl, acetyl, trifluoromethyl, benzyloxy, phenoxy, NH₂, NH(C₁₋₆alkyl) or NH(C₁₋₆alkyl)₂. A preferred optional substituent is a polar substituent. Preferred optional substituents are hydroxy, NH₂ and CO₂H. Examples of alkoxy include methoxy, ethoxy,

n-propoxy, *iso*-propoxy, cyclopropoxy, and butoxy (*n*-, *sec*- *t*- and cyclo) pentoxy and hexyloxy. The "alkyl" portion of an alkoxy group may be substituted as described above.

As used herein, the term "alkenyl" refers to straight, branched, or where appropriate, cyclic carbon containing radicals having one or more double bonds between carbon atoms. Examples of such radicals include vinyl, allyl, butenyl, or longer carbon chains such as those derived from palmitoleic, oleic, linoleic, linolenic or arachidonic acids. An alkenyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO₂, CO₂H, CO₂C₁₋₆alkyl, CONH₂, CONH(C₁₋₆alkyl), CONH(C₁₋₆alkyl)₂, OH, hydroxyalkyl, methyl, ethyl, butyl, propyl, alkoxy, acyl, carboxyalkyl, acetyl, trifluoromethyl, benzyloxy, phenoxy, NH₂, NH(C₁₋₆alkyl) or NH(C₁₋₆alkyl)₂. A preferred optional substituent is a polar substituent, such as NH₂, OH or CO₂H.

As used herein, the term "alkynyl" refers to straight or branched carbon containing radicals having one or more triple bonds between carbon atoms. Examples of such radicals include propargyl, butynyl and hexynyl. An alkynyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO₂, CO₂H, COC₁₋₆alkyl, CO₂NH₂, CONH(C₁₋₆alkyl), CONH(C₁₋₆alkyl)₂, OH, hydroxyalkyl, alkoxy, acyl, carboxyalkyl, acetyl, trifluoromethyl, benzyloxy, phenoxy, NH₂, NH(C₁₋₆alkyl) or NH(C₁₋₆alkyl)₂. A preferred optional substituent is a polar substituent, such as NH₂, OH and CO₂H.

Examples of suitable NH(alkyl) and N(alkyl)₂ include methylamino, ethylamino, isopropylamino, dimethylamino, *n*-propylamino, diethylamino and di-isopropylamino.

The term "halogen" (or "halo") refers to fluorine (fluoro), chlorine (chloro), bromine (bromo) or iodine (iodo).

The term "sugar" refers to a pyranosyl or furanosyl moiety such as those derived from glucose, galactose, mannose, allose, altrose, gulose, idose, talose, ribose, arabinose or xylose. Derivatives of such sugars include deoxy or aminopyranosyl or furanosyl sugar derivatives. Each sugar moiety is incorporated into the compound of formula (I) through a

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hydroxy group of the sugar moiety.

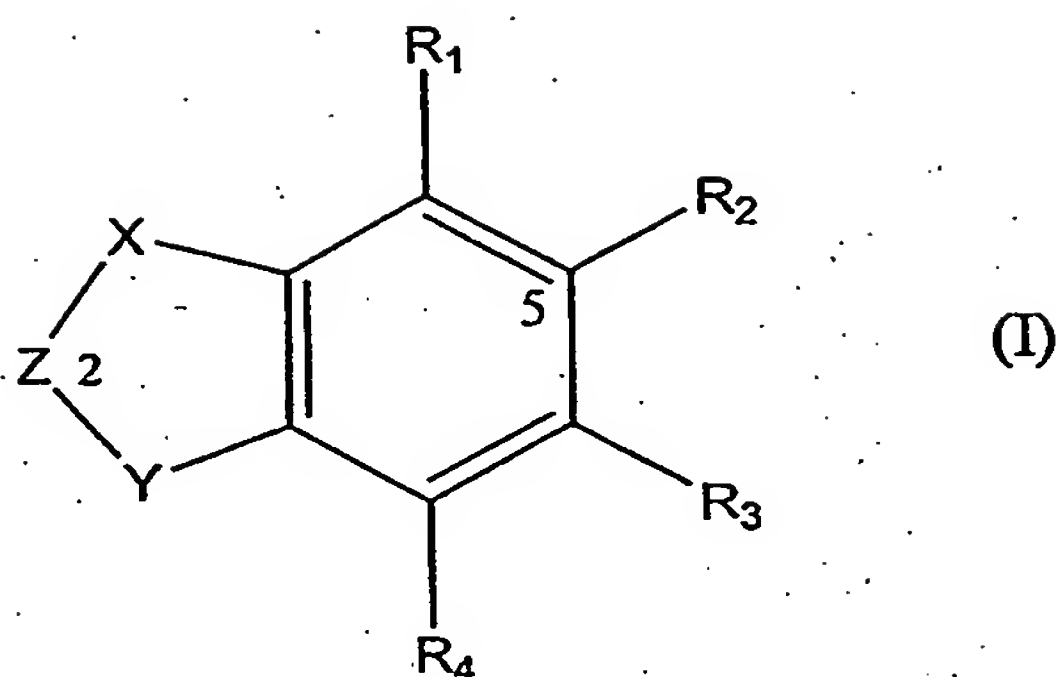
As used herein, "the characterising group of an amino acid" refers to the substituent at C₂ of a naturally occurring or non-naturally occurring amino acid and which defines the amino acid. The amino acid may be in the L- or D-configuration. For example, methyl is the characterising group of alanine, phenylmethyl is the characterising group of phenylalanine, hydroxymethyl is the characterising group of serine, hydroxyethyl is the characterising group of homoserine and n-propyl is the characterising group of norvaline.

10 An aryl group, as used herein, refers to C₆-C₁₀ aryl groups such as phenyl or naphthalene. Aryl groups may be optionally substituted one or more times by halo (eg, chloro, fluoro or bromo), CN, NO₂, CO₂H, CO₂C₁₋₆alkyl, CONH₂, CONH(C₁₋₆alkyl), CONH(C₁₋₆alkyl)₂, OH, hydroxyalkyl, alkoxy, acyl, carboxyalkyl, acetyl, trifluoromethyl, benzyloxy, phenoxy, NH₂, NH(C₁₋₆alkyl) or NH(C₁₋₆alkyl)₂. A preferred optional substituent is a
15 polar substituent, particularly hydroxy, hydroxyalkyl or halo.

As used herein, the term "heterocyclyl" refers to a cyclic, aliphatic or aromatic radical containing at least one heteroatom independently selected from O, N or S. Examples of suitable heterocyclyl groups include furyl, pyridinyl, pyrimidinyl, pyrazolyl, piperidinyl, pyrrolyl, thiophenyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, isothiazolyl, quinolyl, isoquinolyl, indolyl, benzofuranyl, benzothiophenyl, triazolyl, tetrazolyl, oxadiazolyl and purinyl. Heterocyclyl groups may be optionally substituted one or more times by halo (eg, chloro, fluoro or bromo), CN, NO₂, CO₂H, CO₂C₁₋₆alkyl, CONH₂, CONH(C₁₋₆alkyl), CONH(C₁₋₆alkyl)₂, OH, hydroxyalkyl, alkoxy, acyl, carboxyalkyl, acetyl, trifluoromethyl, benzyloxy, phenoxy, NH₂, NH(C₁₋₆alkyl) or NH(C₁₋₆alkyl)₂.
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In a first aspect, the present invention provides a method of inhibiting cytokine or biological activity of MIF comprising contacting MIF with a cytokine or biological activity inhibiting effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof.
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wherein

X is selected from -O-, -S-, -C(R₅)(R₅')- or -N(R₆)- and preferably comprises a hydrogen
5 bond donor or acceptor;

Y is selected from -N(R₇)-, -O-, -S- or -C(R₇)₂-;

Z is selected from -C(O)-, -C(S)-, -C(=NR₆)-, -S(O)- or -S(O)₂-;

10

R₁ is selected from hydrogen, C₁₋₃alkyl, (CR₅R₅')_nOR₇, (CR₅R₅')_nSR₇, (CR₅R₅')_nN(R₆)₂ and
(CR₅R₅')_nhalo;

R₂ is selected from C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R₁₂')_mC(O)R₈,
15 (CR₁₂R₁₂')_mC(S)R₈, (CR₁₂R₁₂')_mS(O)R₈, (CR₁₂R₁₂')_mS(O)₂R₈, (CR₁₂R₁₂')_mOR₉,
(CR₁₂R₁₂')_mSR₉, (CR₁₂R₁₂')_mNR₁₀R₁₁, (CR₁₂R₁₂')_mC(=NR₂₄)R₂₂ and (CR₁₂R₁₂')_mR₁₃;

R₃ is selected from hydrogen, C₁-C₆alkyl, (CR₁₆R₁₆')_pNR₁₄R₁₅, (CR₁₆R₁₆')_pOR₁₇,
(CR₁₆R₁₆')_pSR₁₇, (CR₁₆R₁₆')_phalo, (CR₁₆R₁₆')_pNO₂, (CR₁₆R₁₆')_nC(O)R₂₈,
20 (CR₁₆R₁₆')_nC(=NR₂₄)R₂₂, (CR₁₆R₁₆')_nS(O)R₁₇, (CR₁₆R₁₆')_nS(O)₂R₁₇, (CR₁₆R₁₆')_nS(O)₃R₁₇
and (CR₁₆R₁₆')_pC(R₁₈)₃;

25

R₄ is selected from hydrogen, halogen, C₁-C₃alkyl, C₂₋₃alkenyl, C₂₋₃alkynyl and
(CR₁₂R₁₂')_nC(R₁₈)₃;

Each R₅ and R₅' is independently selected from hydrogen, C₁-C₃alkyl, halo, OR₇, SR₇ and

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$N(R_6)_2$;

Each R_6 is independently selected from hydrogen, C_1 - C_3 alkyl and OR_7 ;

5 Each R_7 is independently selected from hydrogen and C_1 - C_3 alkyl;

R_8 is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, OR_{19} , SR_{19} , $N(R_{20})_2$, $[NH-CH(R_{21})-C(O)]_q-OR_{29}$, $[sugar]_q$ and $(CR_{12}R_{12'})_tR_{13}$;

10 R_9 is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{12}R_{12'})_tR_{13}$, $C(O)R_{23}$, CO_2R_{23} , $C(S)R_{23}$, $C(S)OR_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q-R_{23}$ and $[sugar]_q$;

15 R_{10} and R_{11} are independently selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{12}R_{12'})_mR_{13}$, $C(O)R_{23}$, $C(S)R_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q-R_{23}$, $-[sugar]_q$ and $NHC(=NR_{25})-NH_2$;

Each R_{12} and $R_{12'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, OR_{24} , SR_{24} , halo, $N(R_{24})_2$, CO_2R_{24} , CN , NO_2 , aryl or heterocyclyl;

20

R_{13} is selected from OR_{25} , SR_{25} , halo, $N(R_{25})_2$, $C(O)R_{31}$, CN , $C(R_{18})_3$, aryl or heterocyclyl;

R_{14} and R_{15} are independently selected from hydrogen, C_1 - C_3 alkyl, OR_{17} , $(CR_{16}R_{16'})_pC(R_{18})_3$;

25

Each R_{16} and $R_{16'}$ is independently selected from hydrogen, C_1 - C_3 alkyl, halo, OR_{17} , SR_{17} and $N(R_{17})_2$;

Each R_{17} is independently selected from hydrogen and C_1 - C_3 alkyl;

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Each R_{18} is independently selected from hydrogen and halo;

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R_{19} and each R_{20} are independently selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{26}R_{26'})_tR_{27}$;

5 R_{21} is the characterising group of an amino acid;

R_{22} is selected from C_1 - C_6 alkyl, NH_2 , $NH(C_1$ - C_6 alkyl), $N(C_1$ - C_6 alkyl) $_2$, OR_{29} or SR_{29} ;

10 R_{23} is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, aryl $(CR_{26}R_{26'})_tR_{27}$;

Each R_{24} is independently selected from hydrogen and C_1 - C_6 alkyl;

15 Each R_{25} is independently selected from hydrogen, C_1 - C_6 alkyl, C_1 - C_3 alkoxy C_1 - C_3 alkyl, aryl and heterocyclyl;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, OR_{29} , SR_{29} , halo, $N(R_{29})_2$, CO_2R_{29} , CN , NO_2 , aryl and heterocyclyl;

20 R_{27} is selected from hydrogen, OR_{30} , SR_{30} , halo, $N(R_{30})_2$, CO_2R_{30} , aryl and heterocyclyl;

R_{28} is selected from hydrogen, C_1 - C_6 alkyl, OR_{29} , SR_{29} or $N(R_{29})_2$;

25 Each R_{29} is independently selected from hydrogen and C_1 - C_3 alkyl;

Each R_{30} is independently selected from hydrogen, C_1 - C_3 alkyl, aryl and heterocyclyl;

30 R_{31} is selected from C_1 - C_3 alkyl, OH , C_1 - C_3 alkoxy, aryl, aryloxy, heterocyclyl and heterocyclxyloxy;

n is 0 or an integer from 1 to 3;

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m is 0 or an integer from 1 to 20;

p is 0 or an integer from 1 to 6;

q is an integer from 1 to 5;

t is an integer from 1 to 10;

5

wherein alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

In a preferred embodiment one or more of the following definitions apply:

10 X is -N(H)-, -N(C₁₋₃alkyl)-, -N(OH)-, -N(OC₁₋₃alkyl)-, -O-, -S-, -CH₂-, -CH(OH)-, -CH(NH₂)-, -CH(C₁₋₃alkyl)-, -CH(halo)-, -CH(SH)-, -CH(OC₁₋₃alkyl) or -CH(SC₁₋₃alkyl)-; more preferably, -N(H)-, -CH₂-, -S- or -O-.

Y is -NH-, -N(C₁₋₃alkyl)-, -O-, -S- or -CH₂-; more preferably -O-, -NH- or -CH₂-;

15

Z is -C(O)-, -C(S)-, -S(O)-, -C(=NH)-, -C(=NC₁₋₃alkyl)-, -C(=NOH)- or -C(=NOC₁₋₃alkyl), more preferably -C(O)-, -C(S)- or -S(O)-;

20

R₁ is hydrogen, CH₃, OH, SH, NH₂, NHCH₃, F, Cl or Br, more preferably hydrogen, CH₃, Br or NHCH₃;

25

R₂ is selected from C₁₋₂₀alkyl, C₁₋₂₀alkenyl, (CR₁₂R_{12'})_mheterocyclyl, (CR₁₂R_{12'})_maryl, (CR₁₂R_{12'})_mhalo, (CR₁₂R_{12'})_mOH, (CR₁₂R_{12'})_mOC₁₋₂₀alkyl, (CR₁₂R_{12'})_mOC₂₋₂₀alkenyl, (CR₁₂R_{12'})_mOC(O)C₁₋₂₀alkyl, (CR₁₂R_{12'})_mOC(O)C₂₋₂₀alkenyl, (CR₁₂R_{12'})_mOC(O)aryl, (CR₁₂R_{12'})_mO[C(O)CH(R₂₁)NH]_r-H, (CR₁₂R_{12'})_mO[sugar]_r, (CR₁₂R_{12'})_mNH₂, (CR₁₂R_{12'})_mNHC₁₋₂₀alkyl, (CR₁₂R_{12'})_mN(C₁₋₂₀alkyl)₂, (CR₁₂R_{12'})_mNHC₂₋₂₀alkenyl, (CR₁₂R_{12'})_mN(C₂₋₂₀alkenyl)₂, (CR₁₂R_{12'})_mN(C₁₋₂₀alkyl)(C₂₋₂₀alkenyl), (CR₁₂R_{12'})_mNHC(O)C₁₋₂₀alkyl, (CR₁₂R_{12'})_mNHC(O)C₂₋₂₀alkenyl, (CR₁₂R_{12'})_mNHC(O)aryl, (CR₁₂R_{12'})_mNH[C(O)CH(R₂₁)NH]_r-H, (CR₁₂R_{12'})_mNH-[sugar]_r, (CR₁₂R_{12'})_mSO₃H, (CR₁₂R_{12'})_mSO₃C₁₋₂₀alkyl, (CR₁₂R_{12'})_mSO₃C₂₋₂₀alkenyl, (CR₁₂R_{12'})_mC(O)C₁₋₂₀alkyl, (CR₁₂R_{12'})_mC(O)C₂₋₂₀alkenyl, (CR₁₂R_{12'})_mCO₂H, (CR₁₂R_{12'})_mCO₂C₁₋₂₀alkyl,

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$(\text{CR}_{12}\text{R}_{12'})_m\text{CO}_2\text{C}_{2-20}\text{alkenyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{NHC}_{1-20}\text{alkyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{N}(\text{C}_{1-20}\text{alkyl})_2$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{NHC}_{2-20}\text{alkenyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{N}(\text{C}_{2-20}\text{alkenyl})_2$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{N}(\text{C}_{1-20}\text{alkyl})(\text{C}_{2-20}\text{alkenyl})$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})[\text{NHCH}(\text{R}_{21})\text{C}(\text{O})]_r\text{-OH}$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})[\text{NHCH}(\text{R}_{21})\text{C}(\text{O})]_r\text{-OCH}_3$ $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})[\text{sugar}]_r$, $(\text{CR}_{12}\text{R}_{12'})_m\text{SC}_{1-6}\text{alkyl}$, $\text{C}(\text{=N})\text{NHC}_{1-6}\text{alkyl}$; wherein each R_{12} and $\text{R}_{12'}$ is independently selected from
hydrogen, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$, halogen, OH, hydroxy $\text{C}_{1-6}\text{alkyl}$, $\text{OC}_{1-6}\text{alkyl}$,
 CO_2H , $\text{CO}_2\text{C}_{1-3}\text{alkyl}$, NH_2 , $\text{NHC}_{1-3}\text{alkyl}$, $\text{N}(\text{C}_{1-3}\text{alkyl})_2$, CN, NO_2 , aryl or heterocyclyl; R_{21}
is the characterising group of an amino acid, m is 0 or an integer from 1 to 20 and r is an
integer from 1 to 5; more preferably R_2 is selected from $(\text{CH}_2)_{1-6}\text{CO}_2\text{H}$, $\text{C}_{1-20}\text{alkyl}$, $\text{C}_{1-20}\text{alkenyl}$, $\text{CO}_2\text{C}_{1-6}\text{alkyl}$, $(\text{CH}_2)_{0-6}\text{NH}_2$, $(\text{CH}_2)_{1-6}\text{heterocyclyl}$, $(\text{CH}_2)_{1-6}\text{aryl}$ wherein aryl is
optionally substituted with one or more OH or hydroxy $\text{C}_{1-3}\text{alkyl}$ groups, $\text{CO}_2\text{C}_{1-6}\text{alkyleneOC}_{1-6}\text{alkyl}$ wherein the $\text{C}_{1-6}\text{alkyl}$ is optionally substituted with OH,
 $\text{C}(\text{O})[\text{NHCH}(\text{R}_{21})\text{C}(\text{O})]_r\text{-OH}$, $\text{C}(\text{O})[\text{NHCH}(\text{R}_{21})\text{C}(\text{O})]_r\text{-OCH}_3$, $\text{SO}_3\text{C}_{1-10}\text{alkyl}$, $\text{SO}_2\text{NHC}_{1-10}\text{alkyl}$, $\text{C}(\text{=N})\text{NHC}_{1-6}\text{alkyl}$, $(\text{CH}_2)_{1-3}\text{Osugar}$, $(\text{CH}_2)_{1-3}\text{SC}_{1-6}\text{alkyl}$, $\text{C}(\text{O})\text{C}_{1-6}\text{alkylCO}_2\text{H}$,
 $\text{C}(\text{O})\text{SC}_{1-6}\text{alkyl}$, $\text{NHC}(\text{O})\text{C}_{1-3}\text{alkylSheterocyclyl}$, $\text{C}(\text{O})\text{NH}(\text{CH}_2)_{1-3}\text{aryl}$ wherein aryl is
optionally substituted with one or more OH groups;

R_3 is hydrogen, halogen $\text{C}_{1-6}\text{alkyl}$, $-(\text{CH}_2)_n\text{NH}_2$, $-(\text{CH}_2)_n\text{NO}_2$, $-(\text{CH}_2)_n\text{-OH}$,
 $(\text{CH}_2)_n\text{C}(\text{O})\text{C}_{1-3}\text{alkyl}$, $-(\text{CH}_2)_n\text{-CF}_3$ or $-(\text{CH}_2)_n\text{-SH}$ wherein n is 0 or an integer from 1 to 3;
more preferably R_3 is hydrogen, NH_2 , $(\text{CH}_2)_n\text{C}(\text{O})\text{C}_{1-3}\text{alkyl}$, NO_2 , Br, OH, or CH_3 ;

R_4 is hydrogen, methyl, ethyl, $-\text{CH}_2=\text{CH}_2$, CH_2CF_3 , fluoro, chloro or bromo, more preferably hydrogen or bromo, especially hydrogen.

At least one of R_5 and $\text{R}_{5'}$ is hydrogen in each $(\text{CR}_5\text{R}_{5'})$ and wherein the number of $(\text{CR}_5\text{R}_{5'})$ as designated by n is greater than 2, preferably less than 2 of R_5 and $\text{R}_{5'}$ are other than hydrogen, more preferably, $(\text{CR}_5\text{R}_{5'})_n$ represents an unsubstituted alkylene chain with n designating the number of methylene groups in the chain.

At least one of R_{12} and $\text{R}_{12'}$ is hydrogen in each $(\text{CR}_{12}\text{R}_{12'})$ and wherein the number of $(\text{CR}_{12}\text{R}_{12'})$ as designated by m is greater than 5, preferably less than 5 of R_{12} and $\text{R}_{12'}$ are

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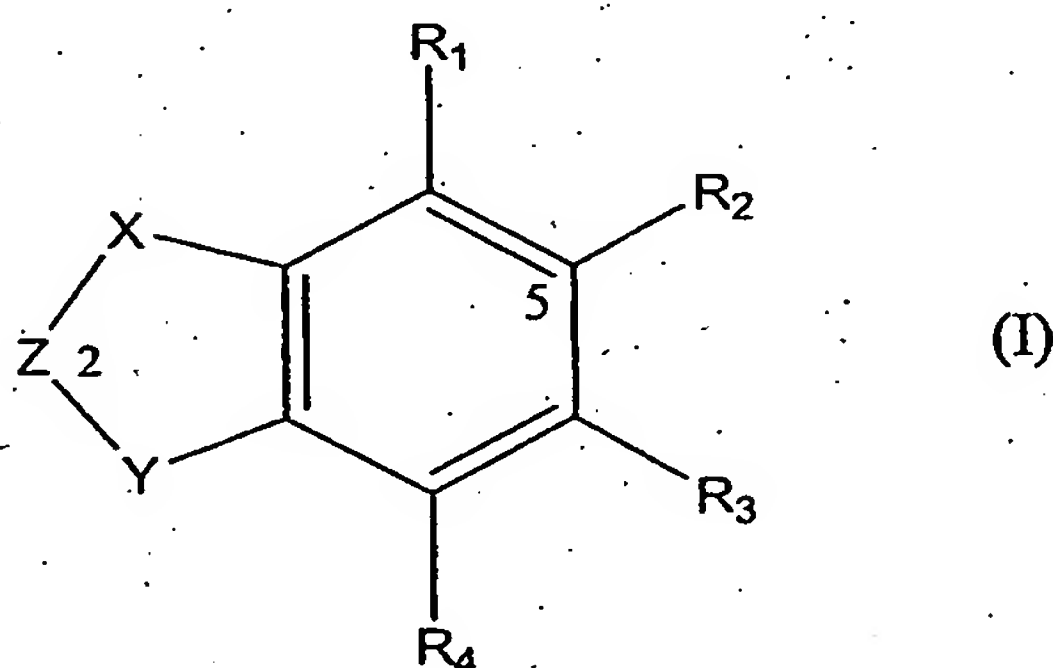
other than hydrogen, more preferably, $(\text{CR}_{12}\text{R}_{12'})_m$ represents an unsubstituted alkylene chain with m designating the number of methylene groups in the chain.

At least one of R_{16} and $\text{R}_{16'}$ is hydrogen in each $(\text{CR}_{16}\text{R}_{16'})$ and wherein the number of $(\text{CR}_{16}\text{R}_{16'})$ as designated by n is greater than 2, preferably less than 2 of R_{16} and $\text{R}_{16'}$ are other than hydrogen, and wherein the number of $(\text{CR}_{16}\text{R}_{16'})$ as designated by p is greater than 4, preferably less than 4 of R_{16} and $\text{R}_{16'}$ are other than hydrogen, more preferably, $(\text{CR}_{16}\text{R}_{16'})_n$ and $(\text{CR}_{16}\text{R}_{16'})_p$ represent an unsubstituted alkylene chain with n or p designating the number of methylene groups in the chain.

At least one of R_{26} and $\text{R}_{26'}$ is hydrogen in each $(\text{CR}_{26}\text{R}_{26'})$ and wherein the number of $(\text{CR}_{26}\text{R}_{26'})$ as designated by t is greater than 5, preferably less than 5 of R_{26} and $\text{R}_{26'}$ are other than hydrogen, more preferably, $(\text{CR}_{26}\text{R}_{26'})_t$ represents an unsubstituted alkylene chain with t designating the number of methylene groups in the chain.

Alkyl, alkenyl, alkynyl, aryl and heterocyclyl, are optionally substituted with one or more substituents selected from the group halogen, hydroxy, hydroxyalkyl, alkoxy, C_{1-6} alkyl, carboxylic acid, carboxylic ester, amino, alkyl substituted amino, $-\text{CN}$ and $-\text{NO}_2$, particularly halogen, hydroxy, hydroxyalkyl and carboxylic acid.

In certain preferred forms of the invention, the compounds of formula (I) include:



wherein

X is selected from $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{R}_5)(\text{R}_5)-$ or $-\text{N}(\text{R}_6)-$ and preferably comprises a hydrogen

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bond donor or acceptor;

Y is selected from $-N(R_7)-$ or $-C(R_7)_2-$;

5 Z is selected from $-C(O)-$, $-C(S)-$, $-C(=NR_6)-$, $-S(O)-$ or $-S(O)_2-$;

R_1 is selected from hydrogen, C_{1-3} alkyl, $(CR_5R_5')_nOR_7$, $(CR_5R_5')_nSR_7$, $(CR_5R_5')_nN(R_6)_2$ and $(CR_5R_5')_n$ halo;

10 R_2 is selected from C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, $(CR_{12}R_{12'})_mC(O)R_8$, $(CR_{12}R_{12'})_mC(S)R_8$, $(CR_{12}R_{12'})_mS(O)R_8$, $(CR_{12}R_{12'})_mS(O)_2R_8$, $(CR_{12}R_{12'})_mOR_9$, $(CR_{12}R_{12'})_mSR_9$, $(CR_{12}R_{12'})_mNR_{10}R_{11}$, $(CR_{12}R_{12'})_mC(=NR_{24})R_{22}$ and $(CR_{12}R_{12'})_mR_{13}$;

R_3 is selected from hydrogen, C_1-C_6 alkyl, $(CR_{16}R_{16'})_pNR_{14}R_{15}$, $(CR_{16}R_{16'})_pOR_{17}$,
15 $(CR_{16}R_{16'})_pSR_{17}$, $(CR_{16}R_{16'})_p$ halo, $(CR_{16}R_{16'})_pNO_2$, $(CR_{16}R_{16'})_nC(O)R_{28}$, $(CR_{16}R_{16'})_nC(=NR_{24})R_{22}$, $(CR_{16}R_{16'})_nS(O)R_{17}$, $(CR_{16}R_{16'})_nS(O)_2R_{17}$, $(CR_{16}R_{16'})_nS(O)_3R_{17}$ and $(CR_{16}R_{16'})_pC(R_{18})_3$;

R_4 is selected from hydrogen, halogen, C_1-C_3 alkyl, C_2-C_3 alkenyl, C_2-C_3 alkynyl and
20 $(CR_{12}R_{12'})_nC(R_{18})_3$;

Each R_5 and R_5' is independently selected from hydrogen, C_1-C_3 alkyl, halo, OR_7 , SR_7 and $N(R_6)_2$;

25 Each R_6 is independently selected from hydrogen, C_1-C_3 alkyl and OR_7 ;

Each R_7 is independently selected from hydrogen and C_1-C_3 alkyl;

R_8 is selected from hydrogen, C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, OR_{19} , SR_{19} ,
30 $N(R_{20})_2$, $[NH-CH(R_{21})-C(O)]_q-OR_{29}$, [sugar]_q and $(CR_{12}R_{12'})_tR_{13}$;

- 24 -

R_9 is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{12}R_{12'})_tR_{13}$, $C(O)R_{23}$, CO_2R_{23} , $C(S)R_{23}$, $C(S)OR_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q-R_{23}$ and $[sugar]_q$;

5 R_{10} and R_{11} are independently selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{12}R_{12'})_mR_{13}$, $C(O)R_{23}$, $C(S)R_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q-R_{23}$, $-[sugar]_q$ and $NHC(=NR_{25})-NH_2$;

10 Each R_{12} and $R_{12'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, OR_{24} , SR_{24} , halo, $N(R_{24})_2$, CO_2R_{24} , CN , NO_2 , aryl or heterocyclyl;

R_{13} is selected from OR_{25} , SR_{25} , halo, $N(R_{25})_2$, $C(O)R_{31}$, CN , $C(R_{18})_3$, aryl or heterocyclyl;

15 R_{14} and R_{15} are independently selected from hydrogen, C_1 - C_3 alkyl, OR_{17} , $(CR_{16}R_{16'})_pC(R_{18})_3$;

Each R_{16} and $R_{16'}$ is independently selected from hydrogen, C_1 - C_3 alkyl, halo, OR_{17} , SR_{17} and $N(R_{17})_2$;

20 Each R_{17} is independently selected from hydrogen and C_1 - C_3 alkyl;

Each R_{18} is independently selected from hydrogen and halo;

25 R_{19} and each R_{20} are independently selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{26}R_{26'})_tR_{27}$;

R_{21} is the characterising group of an amino acid;

30 R_{22} is selected from C_1 - C_6 alkyl, NH_2 , $NH(C_{1-6}alkyl)$, $N(C_{1-6}alkyl)_2$, OR_{29} or SR_{29} ;

R_{23} is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, aryl

- 25 -

$(CR_{26}R_{26'})_tR_{27}$;

Each R_{24} is independently selected from hydrogen and C_1 - C_6 alkyl;

- 5 Each R_{25} is independently selected from hydrogen, C_1 - C_6 alkyl, C_{1-3} alkoxy C_{1-3} alkyl, aryl and heterocyclyl;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, OR_{29} , SR_{29} , halo, $N(R_{29})_2$, CO_2R_{29} , CN, NO_2 , aryl and heterocyclyl;

10

R_{27} is selected from hydrogen, OR_{30} , SR_{30} , halo, $N(R_{30})_2$, CO_2R_{30} , aryl and heterocyclyl;

R_{28} is selected from hydrogen, C_{1-6} alkyl, OR_{29} , SR_{29} or $N(R_{29})_2$;

- 15 Each R_{29} is independently selected from hydrogen and C_1 - C_3 alkyl;

Each R_{30} is independently selected from hydrogen, C_1 - C_3 alkyl, aryl and heterocyclyl;

- 20 R_{31} is selected from C_{1-3} alkyl, OH, C_{1-3} alkoxy, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

n is 0 or an integer from 1 to 3;

m is 0 or an integer from 1 to 20;

p is 0 or an integer from 1 to 6;

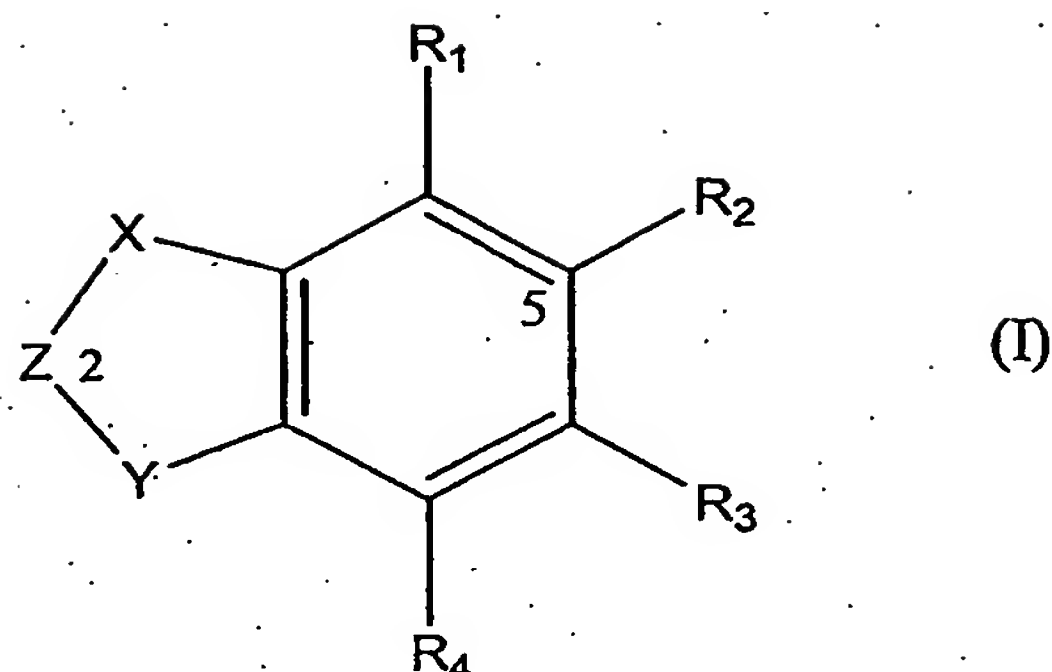
- 25 q is an integer from 1 to 5;

t is an integer from 1 to 10;

wherein alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

- 30 In certain preferred forms of the invention, the compounds of formula (I) include:

- 26 -



wherein

X is -O-, -S-, -C(R₅)₂- or -N(R₆)-;

5

Y is -N(R₇)-, -O-, -S- or -C(R₇)₂-;

Z is -C(O)-, -C(S)-, -S(O)- or -C(=NR₆)-

10 R₁ is hydrogen, CH₃, OH, SH, NH₂, NHCH₃, F, Cl or Br;

R₂ is C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R_{12'})_mC(O)R₈, -(CR₁₂R_{12'})_mC(S)R₈,
 -(CR₁₂R_{12'})_mS(O)R₈, -(CR₁₂R_{12'})_mS(O)₂R₈, -(CR₁₂R_{12'})_mOR₉, -(CR₁₂R_{12'})_mSR₉,
 -(CR₁₂R_{12'})_mNR₁₀R₁₁, (CR₁₂R_{12'})_mC(=NR₂₄)R₂₂ or (CR₁₂R_{12'})_mR₁₃ where m, R₇, R₈, R₉, R₁₀,

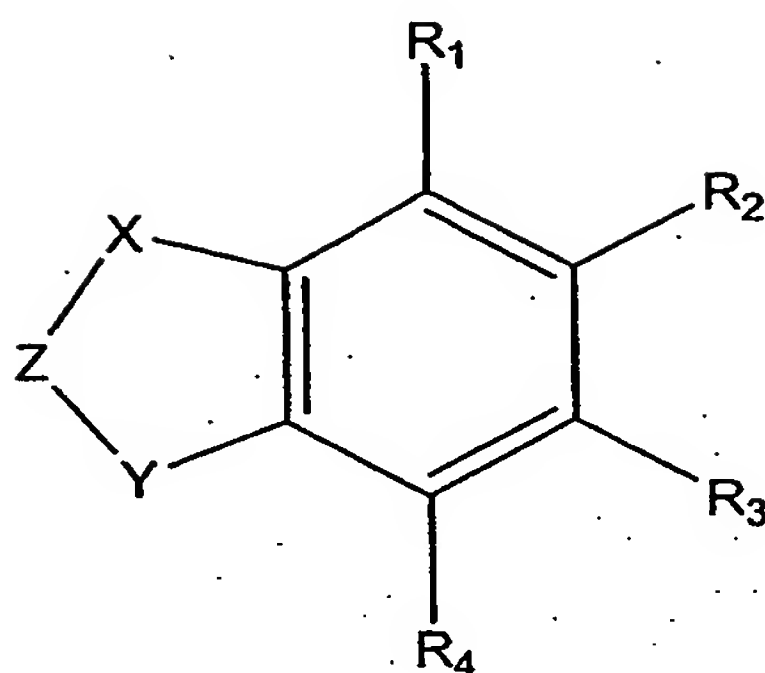
15 R₁₁, R₁₂, R_{12'}, R₁₃, R₂₂ and R₂₄ are defined above;

R₃ is hydrogen, halogen, C₁₋₆alkyl, -(CH₂)_nNH₂, -(CH₂)_nNO₂, -(CH₂)_n-OH, -(CH₂)_nC(O)C₁₋₃alkyl, -(CH₂)_nCF₃ or -(CH₂)_nSH where n is defined above;

20 R₄ is hydrogen, halogen, methyl, ethyl, CH₂CF₃ or -CH₂=CH₂.

Preferred compounds of formula (I) comprise

- 27 -



wherein

X is -N(R₆)-;

5

Y is -N(R₇)- or -C(R₇)₂-;

Z is -C(O)-, -C(S)-, -S(O)- or -C(=NH);

10 R₁ is hydrogen, CH₃, NH₂, NHCH₃, F, Cl or Br;

R₂ is as defined for R₂ above;

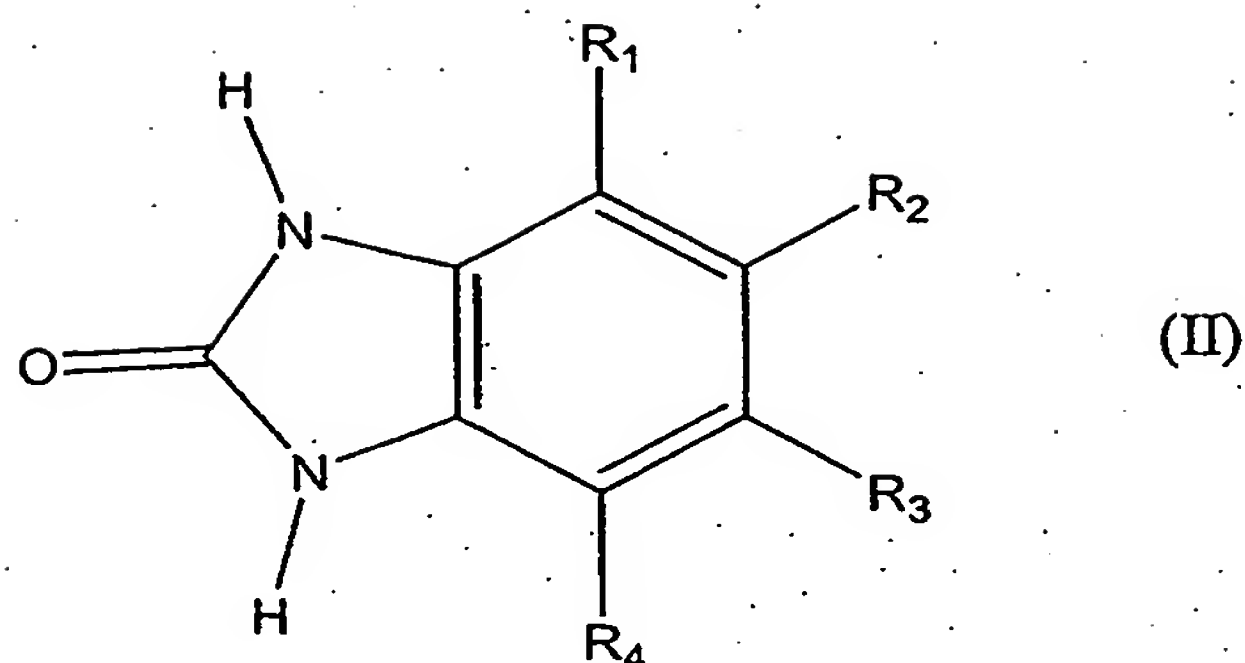
R₃ is hydrogen, halogen, C₁₋₃alkyl, (CH₂)_nNH₂, (CH₂)_nNO₂, (CH₂)_nOH, (CH₂)_nC(O)CH₃ or
 15 (CH₂)_nCF₃ where n is defined above;

R₄ is hydrogen, halogen, methyl, ethyl, CH₂CF₃ or -CH₂=CH₂.

Preferred compounds of formula (I) are benzimidazole compounds having the formula (II)

20

- 28 -



wherein

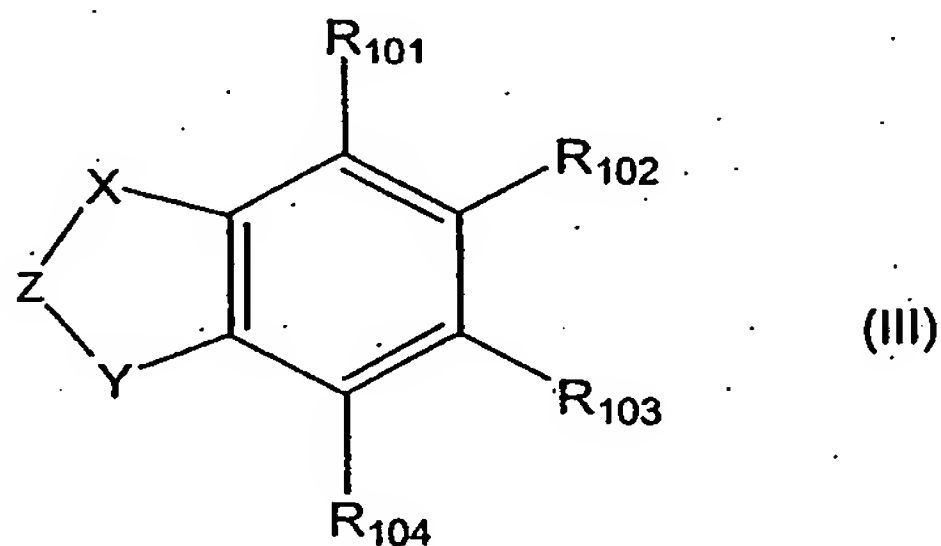
R₁ is hydrogen, CH₃, NHCH₃, F, Cl or Br;

R₂ is as defined for R₂ above;

R₃ is hydrogen, halogen, C₁-C₃alkyl, (CH₂)_nNH₂, (CH₂)_nNO₂, (CH₂)_nOH, CH₂C(O)CH₃, (CH₂)_nCF₃ where n is defined above.

R₄ is hydrogen, halogen, methyl, ethyl, CH₂CF₃ or -CH₂=CH₂.

Other preferred compounds of formula (I) are compounds having formula (III):



wherein

X is -O-, -NH- or -CH₂-;

Y is -NH-, -O- or -CH₂-;

- 29 -

Z is $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$ or $-\text{S}(\text{O})-$;

R_{101} is selected from hydrogen, C_{1-3} alkyl, OH, SH, NH_2 , NHC_{1-3} alkyl, F, Cl or Br;

5

R_{102} is selected from C_{1-20} alkyl, C_{2-20} alkenyl, CO_2H , $\text{CO}_2\text{R}_{105}$, $-\text{NH}_2$, F, Cl, Br, $(\text{CH}_2)_w\text{R}_{106}$, $\text{C}(\text{O})\text{N}(\text{R}_{107})_2$, $\text{C}(=\text{N})\text{NHC}_{1-6}$ alkyl, $\text{SO}_2\text{C}_{1-6}$ alkyl, $\text{C}(\text{O})[\text{NHCH}(\text{R}_{108})\text{C}(\text{O})]_q-\text{OR}_{109}$, $\text{C}(\text{O})\text{sugar}$, $\text{CONH}(\text{CH}_2)_n\text{aryl}$, $\text{NHC}(\text{O})(\text{CH}_2)_n\text{heterocyclyl}$, $\text{C}(\text{O})\text{SC}_{1-6}$ alkyl, $\text{C}(\text{O})(\text{CH}_2)_n\text{CO}_2\text{H}$, $\text{SO}_2\text{OC}_{1-10}$ alkyl, and $\text{SO}_2\text{NHC}_{1-10}$ alkyl;

10

R_{103} is selected from hydrogen, F, Cl, Br, C_{1-6} alkyl, $-(\text{CH}_2)_n\text{NH}_2$, $-(\text{CH}_2)_n\text{NO}_2$, $-(\text{CH}_2)_n\text{OH}$, $-(\text{CH}_2)_n\text{CF}_3$, $-(\text{CH}_2)_n\text{C}(\text{O})\text{C}_{1-3}$ alkyl or $-(\text{CH}_2)_n\text{SH}$;

R_{104} is selected from hydrogen, methyl, ethyl, $\text{CH}_2\text{C}(\text{R}_{110})_3$, $\text{C}(\text{R}_{110})_3$, $-\text{CH}_2=\text{CH}_2$, fluoro, chloro or bromo;

15

R_{105} is selected from hydrogen, C_{1-20} alkyl, C_{2-20} alkenyl or $(\text{CH}_2)_t\text{OC}_{1-3}$ alkyl;

R_{106} is selected from SH, SC_{1-6} alkyl, OH, OC_{1-6} alkyl, sugar, CO_2H , NH_2 , heterocyclyl or aryl;

20

Each R_{107} is independently selected from hydrogen, C_{1-20} alkyl, C_{2-20} alkenyl, $(\text{CH}_2)_t\text{aryl}$ and $(\text{CH}_2)_t\text{heterocyclyl}$;

25 R_{108} is the characterising group of an amino acid;

R_{109} is hydrogen, C_{1-3} alkyl;

Each R_{110} is independently selected from hydrogen and halo; and

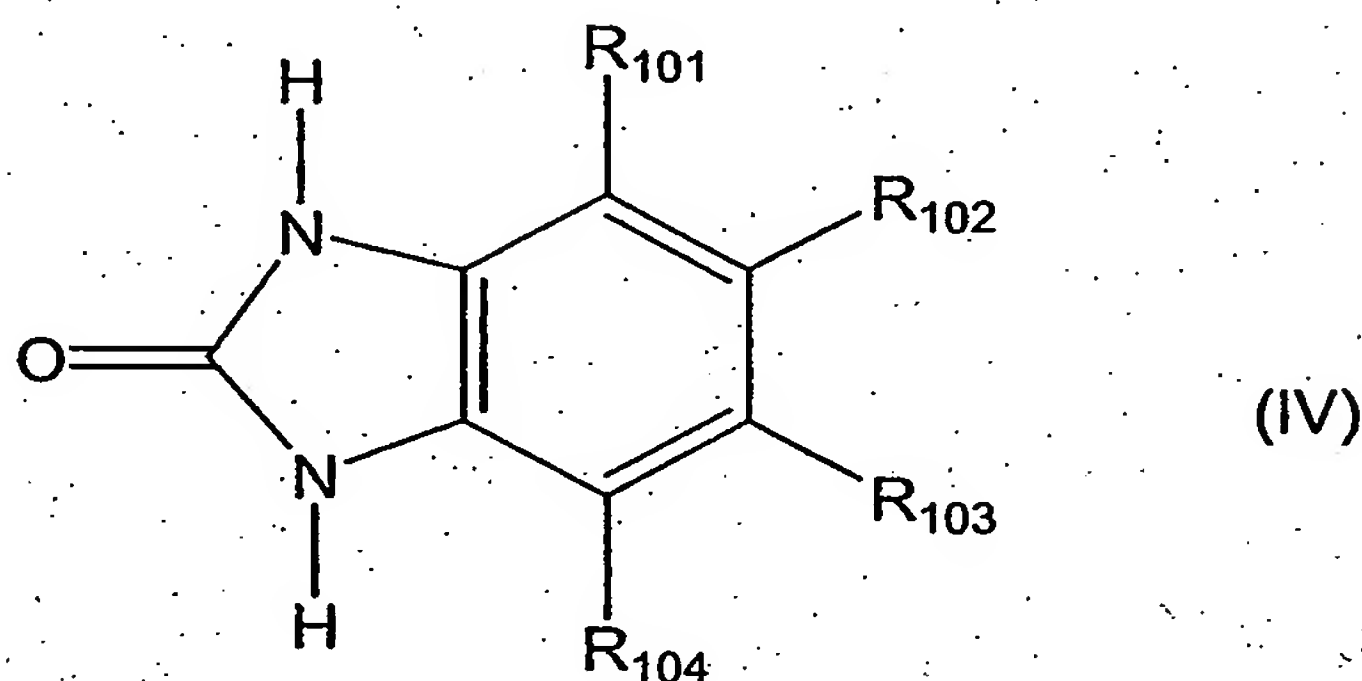
30

n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6; t is

- 30 -

an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

5 Preferred compounds of formula (I) are benzimidazole compounds having formula (IV):



wherein

10

R_{101} is selected from hydrogen, CH_3 , OH, SH, NH_2 , $NHCH_3$, F, Cl or Br;

R_{102} is selected from C_{1-20} alkyl, C_{2-20} alkenyl, CO_2H , CO_2R_{105} , $-NH_2$, F, Cl, Br, $(CH_2)_wR_{106}$, $C(O)N(R_{107})_2$, $C(=N)NHC_{1-6}$ alkyl, SO_2C_{1-6} alkyl, $C(O)[NHCH(R_{108})C(O)]_qOR_{109}$,
 15 $C(O)sugar$, $CONH(CH_2)_naryl$, $NHC(O)(CH_2)_nSheterocyclyl$, $C(O)SC_{1-6}$ alkyl, $C(O)(CH_2)_nCO_2H$, SO_2OC_{1-10} alkyl, and SO_2NHC_{1-10} alkyl;

20

R_{103} is selected from hydrogen, F, Cl, Br, C_{1-6} alkyl, $(CH_2)_nNH_2$, $-(CH_2)_nNO_2$, $-(CH_2)_n-OH$, $-(CH_2)_n-CF_3$, $CH_2C(O)CH_3$ or $-(CH_2)_n-SH$;

R_{104} is selected from hydrogen, methyl, ethyl, CH_2CF_3 , $-CH_2=CH_2$ fluoro, chloro or bromo;

R_{105} is selected from hydrogen, C_{1-10} alkyl, C_{2-10} alkenyl, $(CH_2)_lOC_{1-3}$ alkyl;

25

- 31 -

R_{106} is selected from SH, SC_{1-6} alkyl, OH, OC_{1-6} alkyl, sugar, CO_2H , NH_2 , heterocyclyl or aryl;

Each R_{107} is independently selected from hydrogen, C_{1-10} alkyl, C_{2-10} alkenyl, $(CH_2)_t$ aryl and
5 $(CH_2)_t$ heterocyclyl;

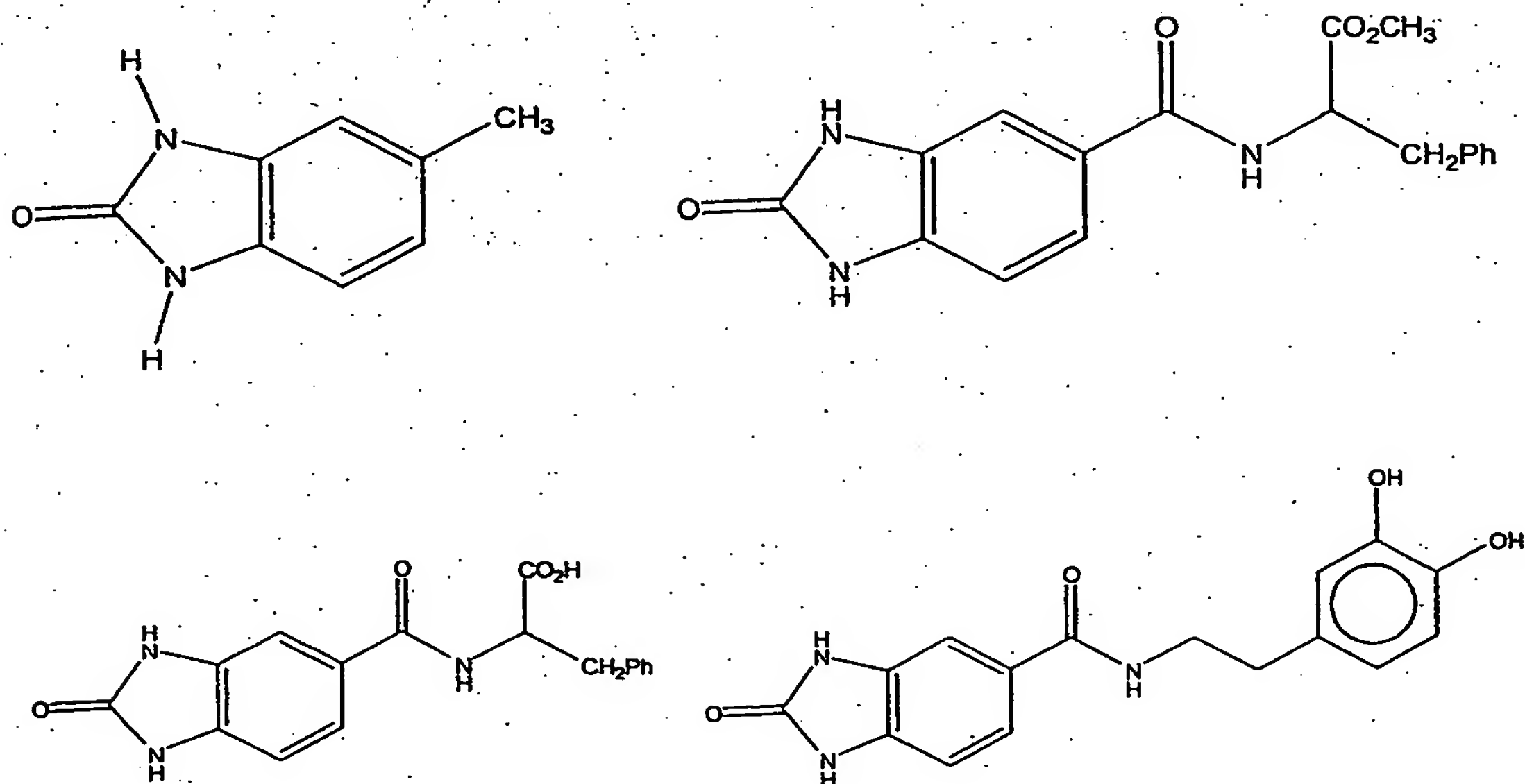
R_{108} is the characterising group of an amino acid;

R_{109} is hydrogen, C_{1-3} alkyl;
10

Each R_{110} is independently selected from hydrogen and halo; and

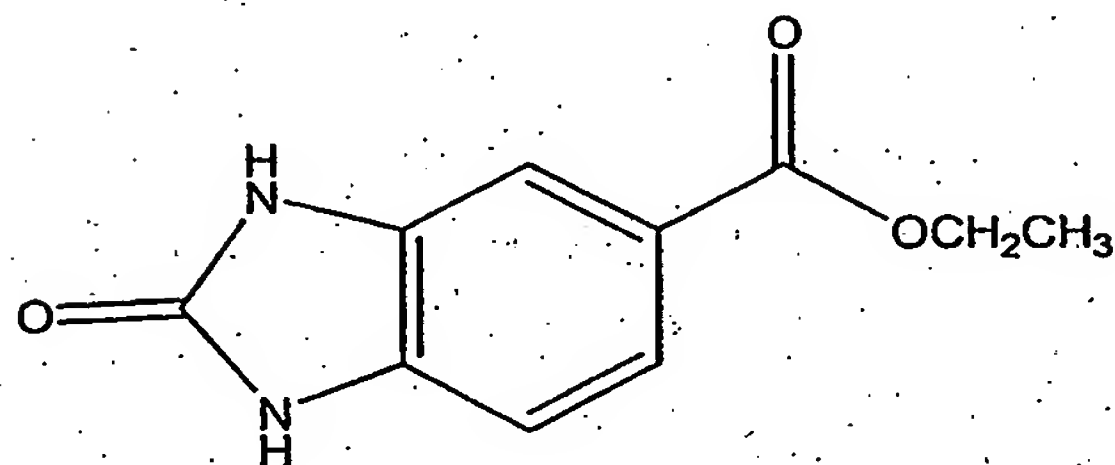
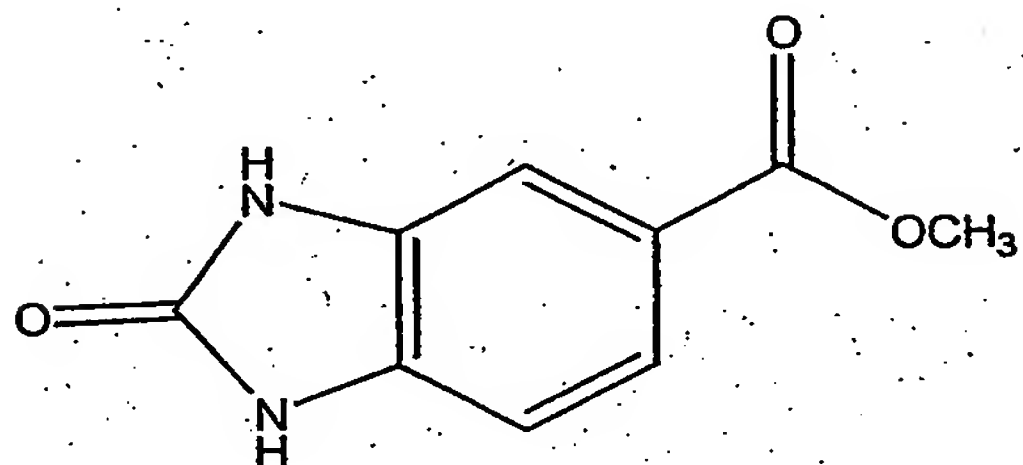
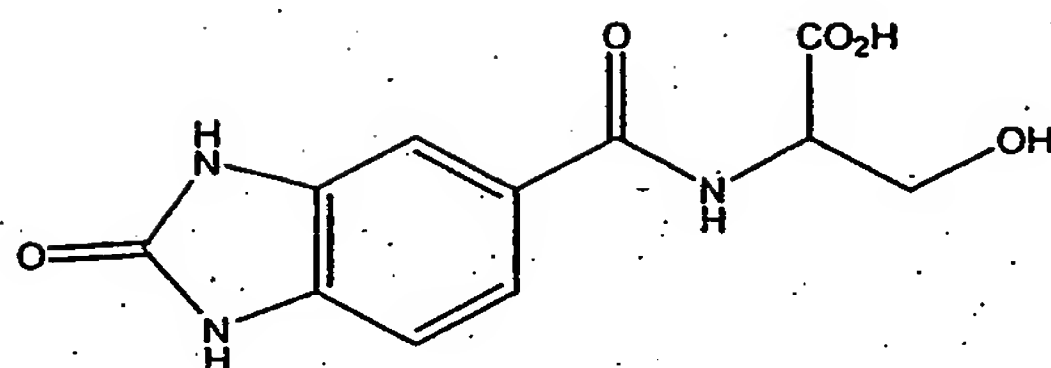
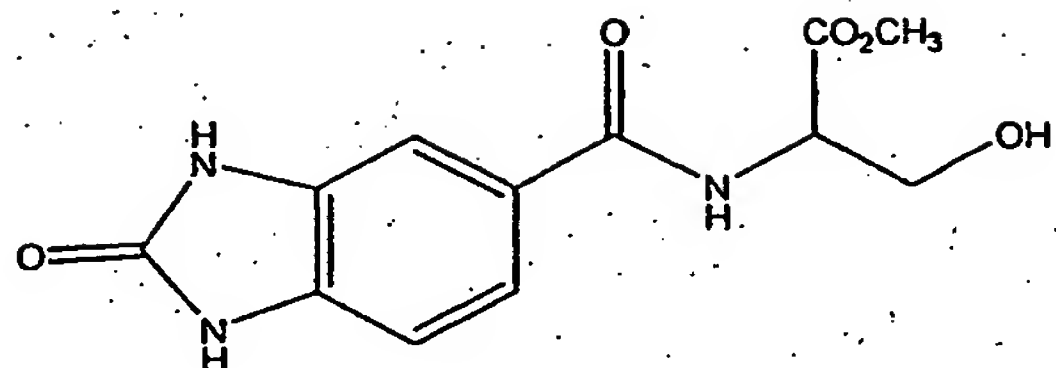
n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6, t is
15 an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

Examples of suitable compound include

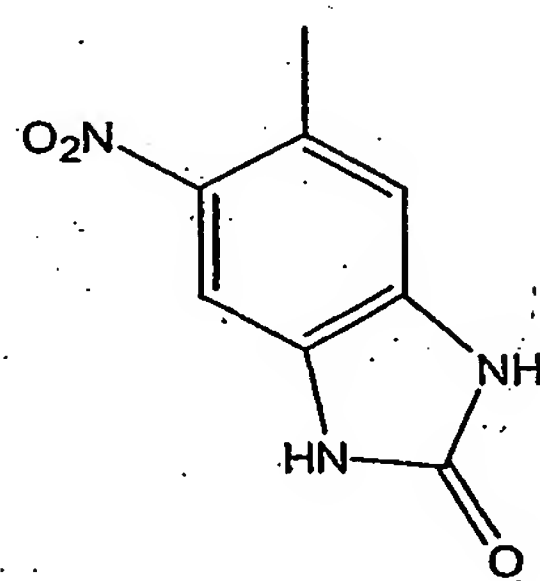
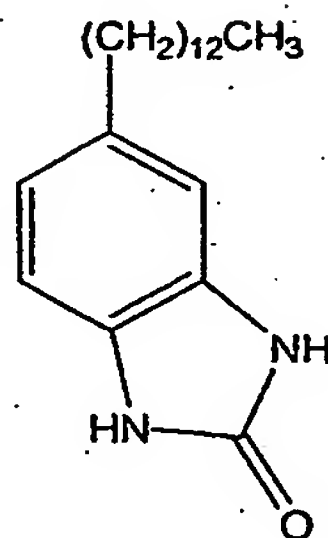
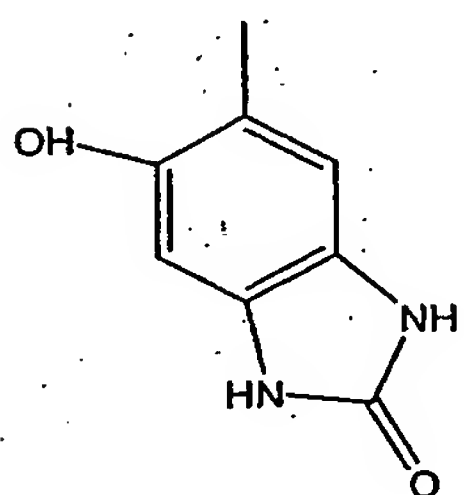
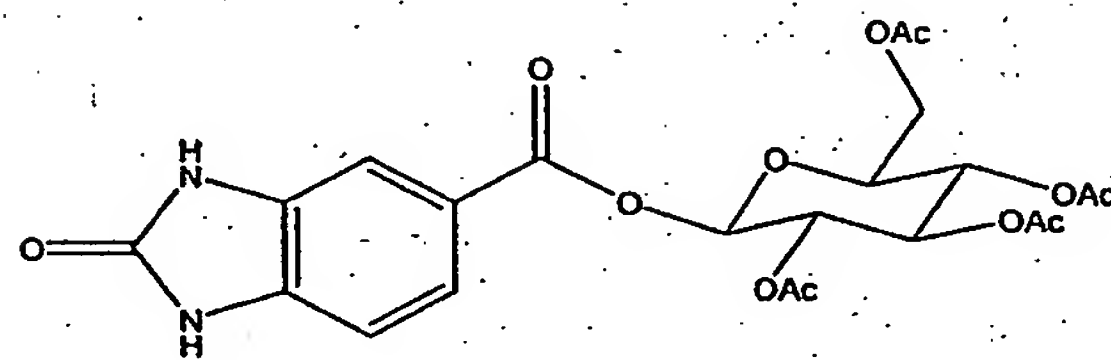
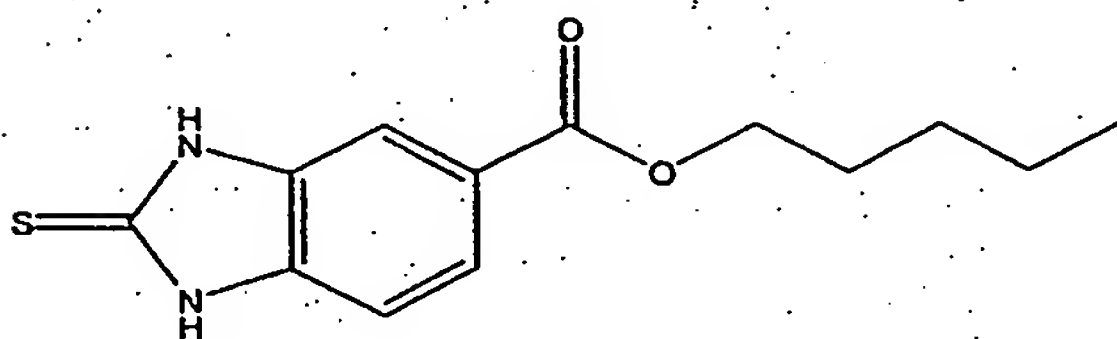
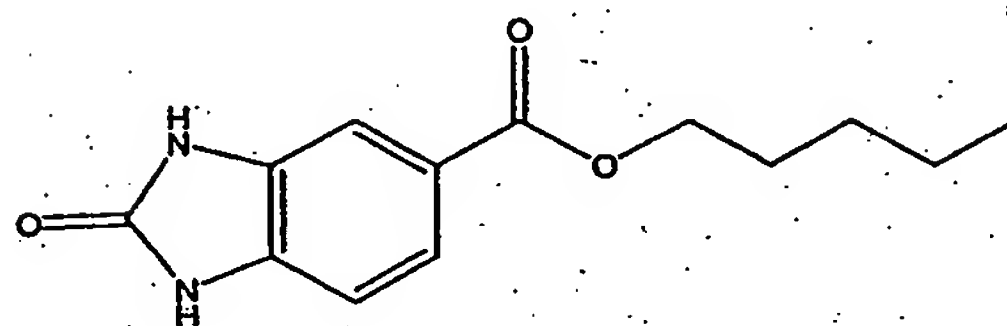
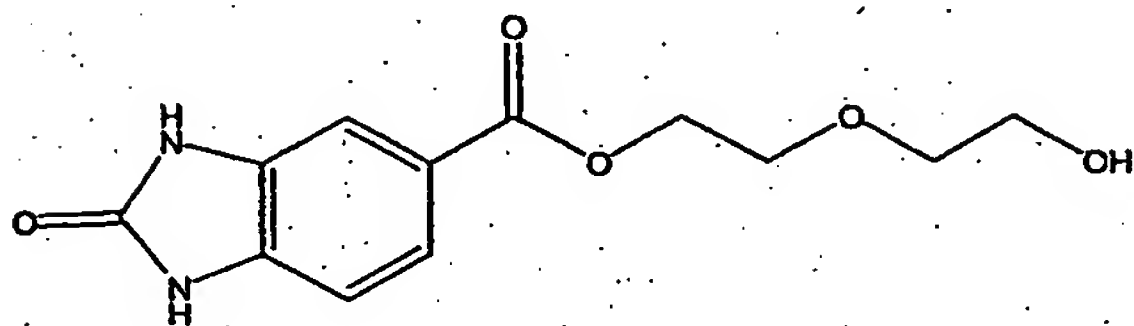


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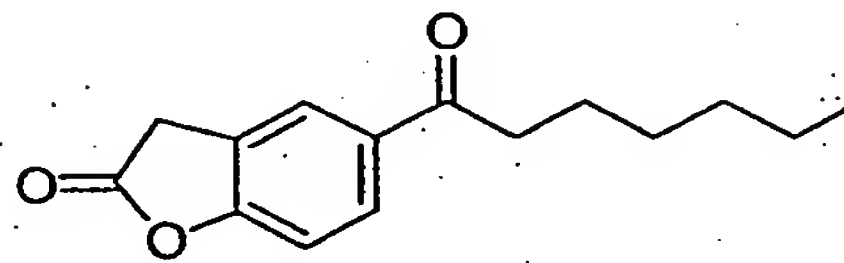
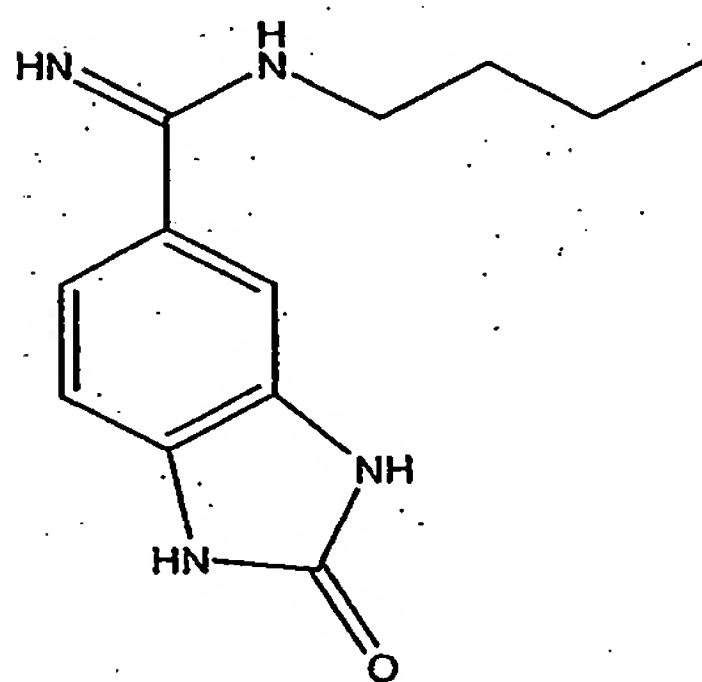
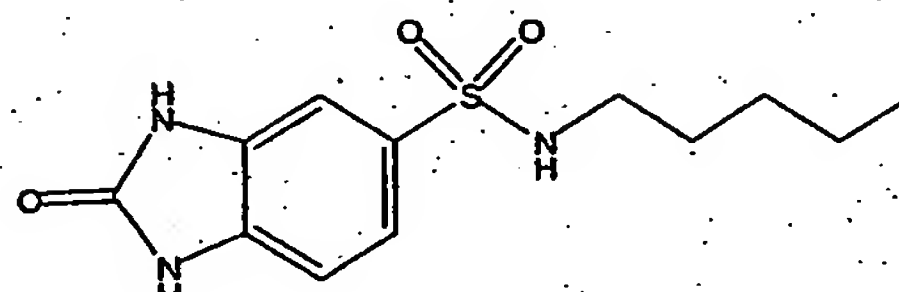
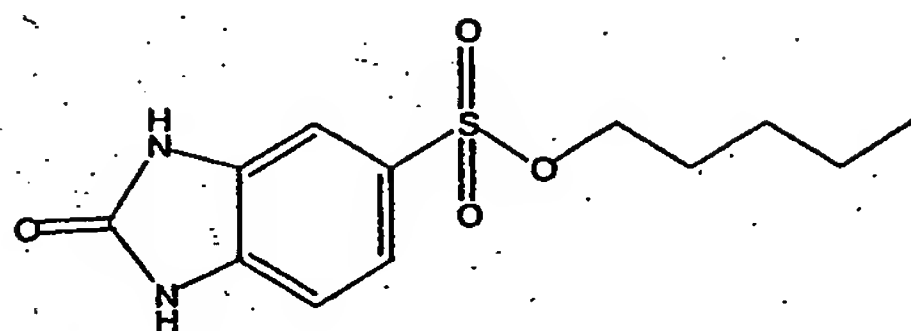
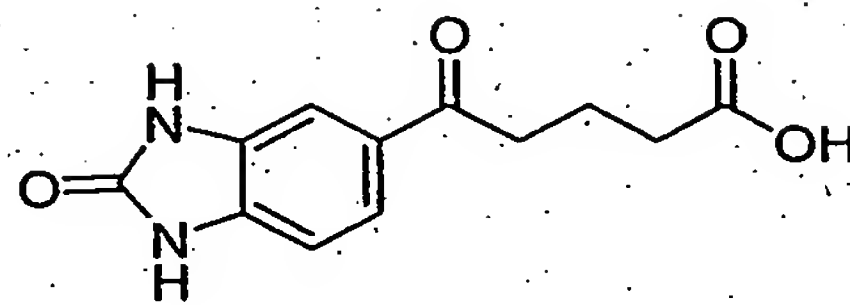
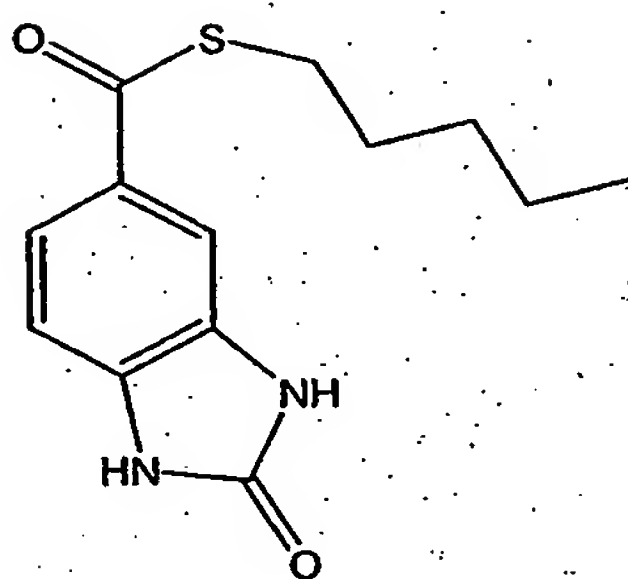
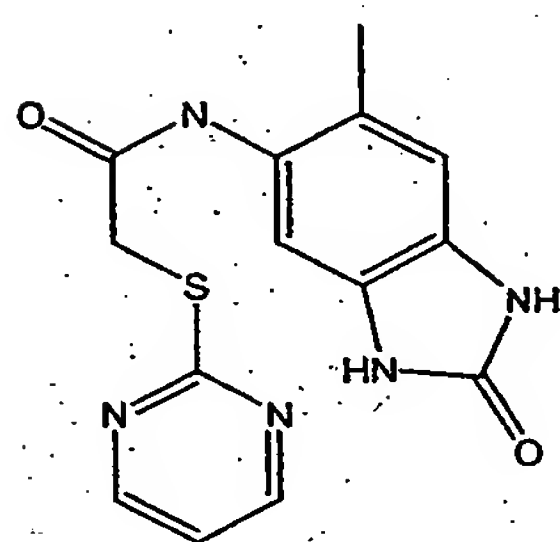
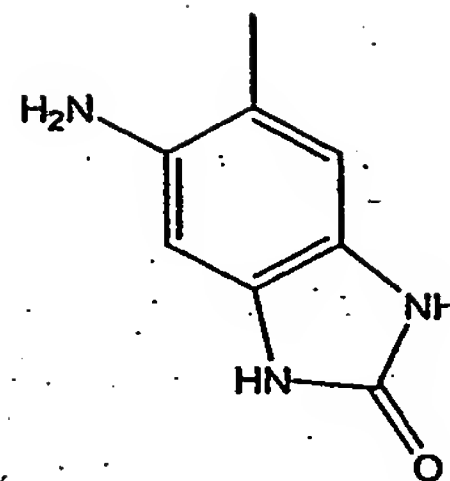
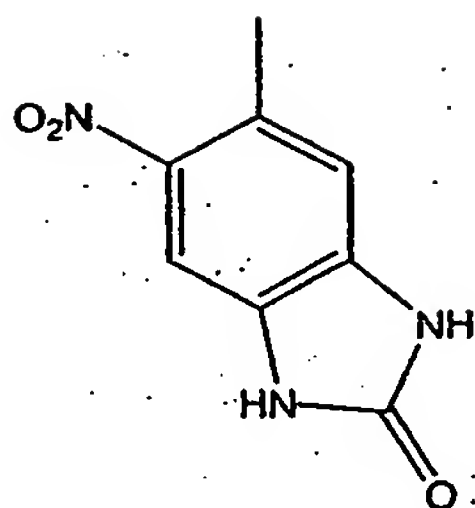
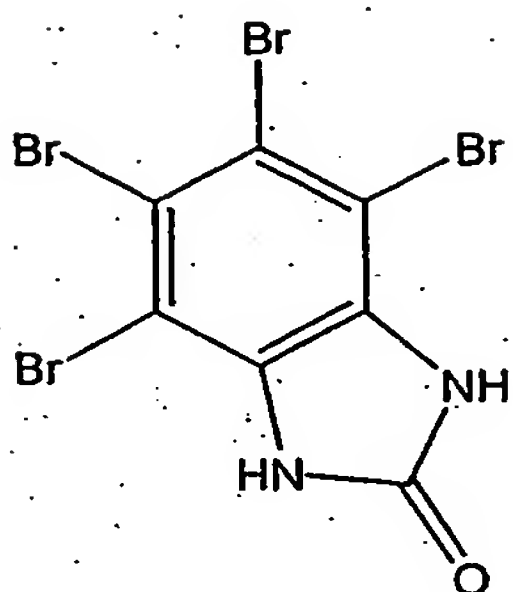


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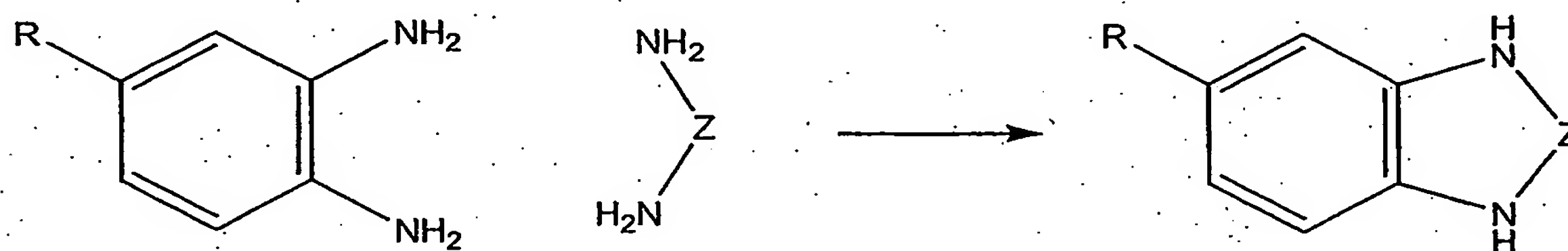


Compounds of formula (I) may be prepared using the methods depicted or described herein
10 or known in the art. It will be understood that minor modifications to methods described
herein or known in the art may be required to synthesize particular compounds of formula
(I). General synthetic procedures applicable to the synthesis of compounds may be found
in standard references such as *Comprehensive Organic Transformations*, R. C. Larock,

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1989, VCH Publishers and *Advanced Organic Chemistry*, J. March, 4th Edition (1992), Wiley InterScience, and references therein. It will also be recognised that certain reactive groups may require protection and deprotection during the synthetic process. Suitable protecting and deprotecting methods for reactive functional groups are known in the art for example in *Protective Groups in Organic Synthesis*, T. W. Green & P. Wutz, John Wiley & Son, 3rd Edition, 1999.

Thus, for certain embodiments of the invention, compounds of formula (I), where X and Y are N and Z is -C(O)-, -S(O)- or -(C=NR₆)- may be prepared in accordance with the exemplified general methods depicted in scheme 1 (3). Suitable starting materials can be obtained commercially or prepared using methods known in the art.

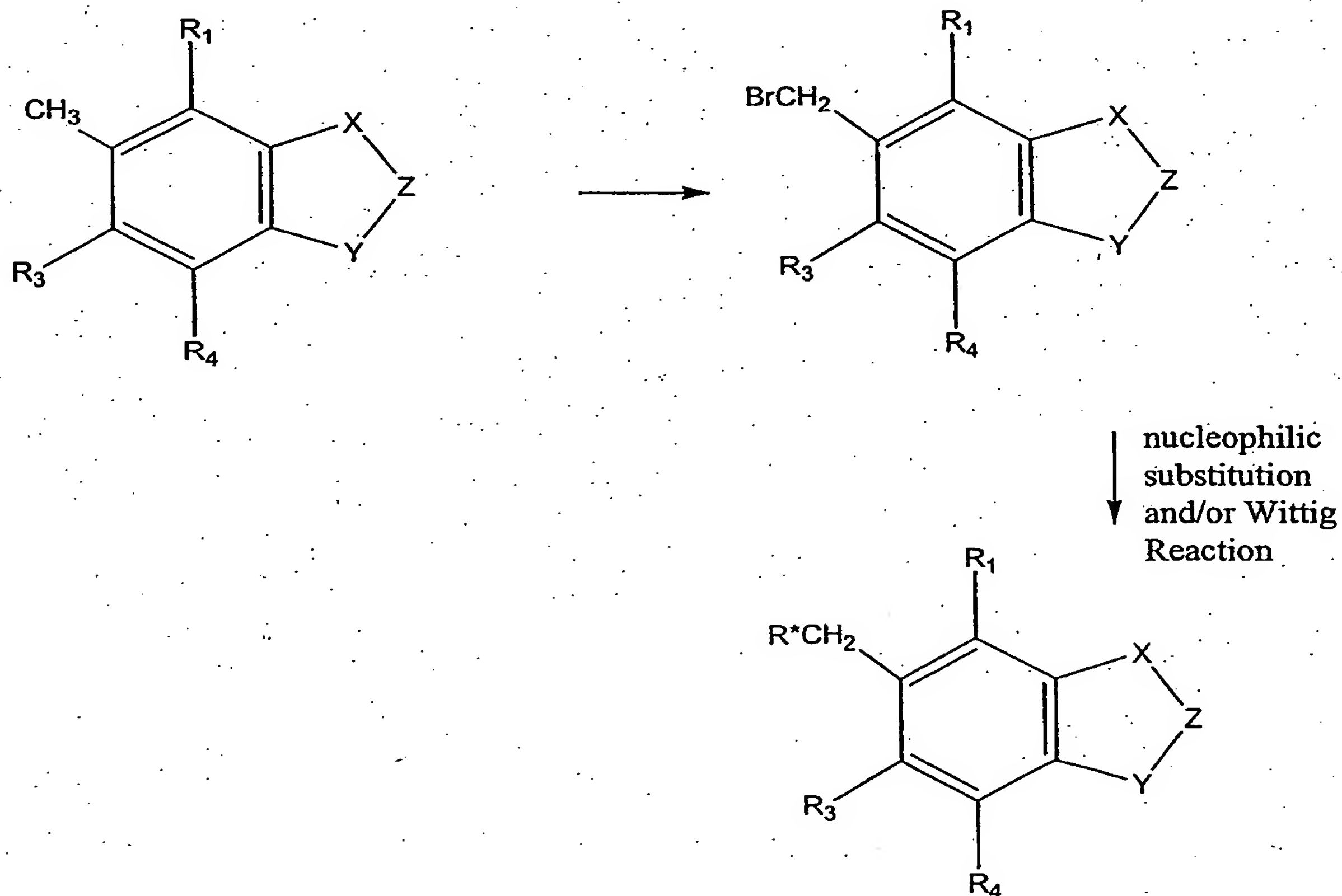


Scheme 1

When R is -CO₂H or -C(S)OH, the compounds may be further derivatised to provide ketones, thioketones, esters, thioesters, amides and thioamides by standard alkylating, esterifying or amide forming methodology. When R is hydroxy, thiol or amino, these groups may be further derivatised to provide esters, thioesters, amides, ethers, thioethers and N-alkyl groups using standard acylating or alkylating methodology. Conversion of an amide to C=NH(NH₂) can be achieved by aminolysis eg NH₃/dry methanol.

When R is CO₂H, a methylene group can be inserted between the benzene nucleus and the carboxylic acid group by Arndt-Eistert synthesis, eg by conversion of the carboxylic acid to an acyl halide and conversion to the diazoketone. Rearrangement of the diazoketone (eg with silver oxide and water) affords access to the CH₂-CO₂H group. Repeating these steps allows for further incorporation of methylene groups. The CO₂H group can be converted as above.

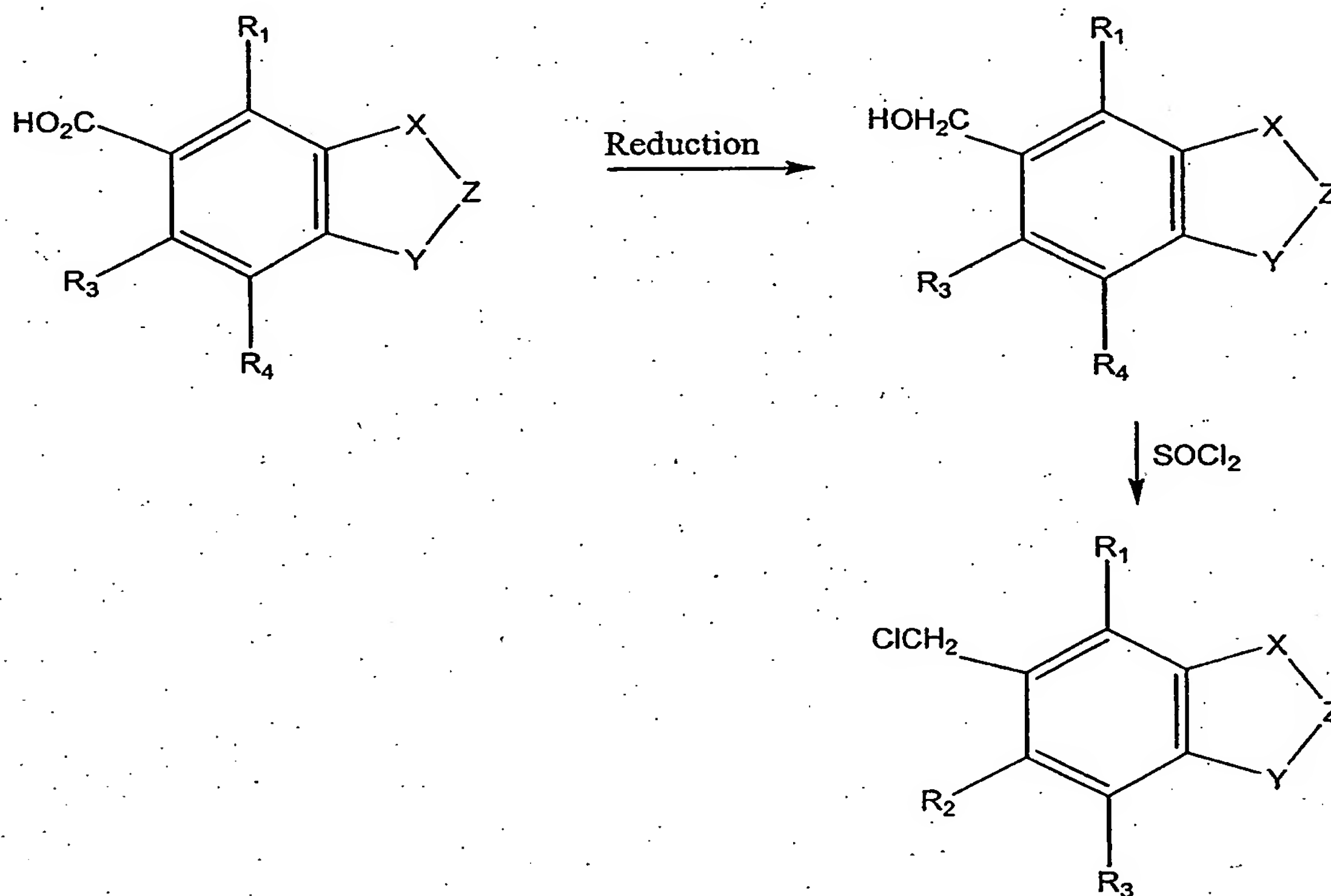
In other embodiments, compounds of formula (I), where R_2 is a substituted methyl group, can be prepared by conversion of the methyl substituent (R_2) into a halomethyl substituent (eg by treatment with a N-halosuccinimide such as NBS) followed by nucleophilic substitution by an appropriate nucleophile and/or insertion of additional methylene groups by, for example, Wittig reaction (see Scheme 2 where R^* can be, for example, $(CH_2)_mOH$, $(CH_2)_mSH$, $(CH_2)_mNH_2$, $(CH_2)_mC(O)C_{1-20}alkyl$, $(CH_2)_mOC(O)C_{1-10}alkyl$, $(CH_2)_mOC_{1-20}alkyl$, $(CH_2)_mOphenyl$, $(CH_2)_mObenzyl$, $(CH_2)_mNHC_{1-20}alkyl$, $(CH_2)_mN(C_{1-20}alkyl)_2$, $(CH_2)_mNHphenyl$, $(CH_2)_mNHbenzyl$, $(CH_2)_mSC_{1-20}alkyl$, $(CH_2)_mSC(O)C_{1-10}alkyl$, $(CH_2)_mSphenyl$, $(CH_2)_mSbenzyl$, $(CH_2)_mNhsugar$, $(CH_2)_mSsugar$, $(CH_2)_mOsugar$, $(CH_2)_mNHC(O)C_{1-10}alkyl$, $(CH_2)_mNHC(O)phenyl$, $(CH_2)_mNHC(O)benzyl$, $(CH_2)_mNHCO_2C_{1-6}alkyl$, $(CH_2)_mNHCO_2phenyl$, or $(CH_2)_mNHCO_2benzyl$, where m is 0 or 1 to 20).



Scheme 2

In other embodiments, compounds where R_2 is CH_2halo can be prepared by reaction of a suitable carboxylic acid derivative with a reducing agent such as LiAlH_4 , followed by halogenation, eg treatment with thionyl chloride (Scheme 3).

5

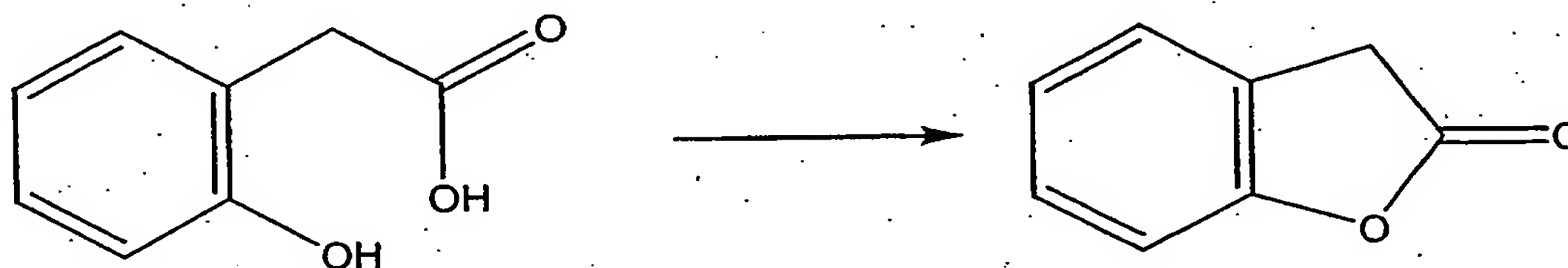


Scheme 3

Coupling of compounds wherein R_2 is CH_2halo with a C_{1-6} alkylhalide, halo(CH_2) $_n$ heterocyclyl in the presence of CuLi affords the corresponding compounds where the R_2 substituent is C_{1-6} alkyl, (CH_2) $_n$ heterocyclyl.

Reaction of CH_2halo with $\text{NH}_2\text{-NH-C(=NH)-NH}_2$ in the presence of base affords access to compounds wherein R_2 is $\text{CH}_2\text{-NH-NH-C(=NH)-NH}_2$. Alternatively, reaction of the CH_2halo group with halo(CH_2) $_p\text{NH-NH-C(=NH)-NH}_2$ (where p is 1 or 2), affords the group (CH_2) $_p\text{NH-NH-C(=NH)-NH}_2$ where p is 2 or 3.

Other embodiments of formula (I) may be prepared by known methods. For example, furan, thiophene and indole derivatives may be prepared by cyclisation of hydroxy acids, thiol acids or amino acids respectively. For example,



Scheme 4

The term "salt, or prodrug" includes any pharmaceutically acceptable salt, ester, solvate, hydrate or any other compound which, upon administration to the recipient is capable of providing (directly or indirectly) a compound of formula (I) as described herein. The term "pro-drug" is used in its broadest sense and encompasses those derivatives that are converted *in vivo* to the compounds of the invention. Such derivatives would readily occur to those skilled in the art, and include, for example, compounds where a free hydroxy group is converted into an ester, such as an acetate, or where a free amino group is converted into an amide. Procedures for acylating hydroxy or amino groups of the compounds of the invention are well known in the art and may include treatment of the compound with an appropriate carboxylic acid, anhydride or acylchloride in the presence of a suitable catalyst or base.

Suitable pharmaceutically acceptable salts include, but are not limited to, salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, salicylic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

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Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium.

- 5 Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

It will also be recognised that some compounds of formula (I) may possess asymmetric
10 centres and are therefore capable of existing in more than one stereoisomeric form. The invention thus also relates to compounds in substantially pure isomeric form at one or more asymmetric centres eg., greater than about 90% ee, such as about 95% or 97% ee or greater than 99% ee, as well as mixtures, including racemic mixtures, thereof. Such isomers may be prepared by asymmetric synthesis, for example using chiral intermediates,
15 or by chiral resolution.

In another aspect, the invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine or biological activity is implicated comprising the administration of a treatment, prevention or diagnostic effective amount of a compound
20 of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In a further aspect, there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament
25 for the treatment, prevention or diagnosis of a disease or condition wherein MIF cytokine or biological activity is implicated.

In a further aspect of the invention there is provided an agent for the treatment, prevention or diagnosis of a disease or condition wherein MIF cytokine or biological activity is
30 implicated comprising a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

As used herein, MIF includes human or other animal MIF and derivatives and naturally occurring variants thereof which at least partially retain MIF cytokine or biological activity. Thus, the subject to be treated may be human or other animal such as a mammal.

- 5 Non-human subjects include, but are not limited to primates, livestock animals (eg sheep, cows, horses, pigs, goats), domestic animals (eg dogs, cats), birds and laboratory test animals (eg mice rats, guinea pigs, rabbits). MIF is also expressed in plants (thus "MIF" may also refer to plant MIF) and where appropriate, compounds of formula (I) may be used in botanical/agricultural applications such as crop control.

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Reference herein to "cytokine or biological activity" of MIF includes the cytokine or biological effect on cellular function via autocrine, endocrine, paracrine, cytokine, hormone or growth factor activity or via intracellular effects.

- 15 In particular, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, solid or haemopoietic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

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Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis),

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pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg dementia, Alzheimer's disease, multiple sclerosis, demyelinating diseases), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, parturition, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing,

comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In a preferred embodiment, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, solid or haemopoietic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), connective tissue diseases (including but not limited to systemic lupus

erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, asthma, bronchitis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction), brain disorders (eg multiple sclerosis, demyelinating diseases), iritis, iridocyclitis, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (including but not limited to osteoporosis, Paget's disease), atopic dermatitis, malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, and wound healing, comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In yet another preferred embodiment of the invention there is provided a method of treating, diagnosing or preventing autoimmune diseases, solid or haemopoietic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's

disease), liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis), pulmonary diseases (including but not limited to asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction), brain disorders (eg multiple sclerosis, demyelinating diseases), uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, transplant rejection, allergies, allergic rhinitis, bone diseases (including but not limited to osteoporosis, Paget's disease), atopic dermatitis, malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, and wound healing, comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In yet another preferred embodiment, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, solid or haemopoietic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis), connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis), pulmonary diseases (including but not limited to asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction), brain disorders (eg

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multiple sclerosis, demyelinating diseases), psoriasis, transplant rejection, allergies, allergic rhinitis, atopic dermatitis, and wound healing, comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

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In a further preferred embodiment, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis,), connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis,), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis,), pulmonary diseases (including but not limited to asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome), atherosclerosis (eg ischaemic heart disease, myocardial infarction), brain disorders (eg multiple sclerosis, demyelinating diseases), psoriasis, transplant rejection, allergies, allergic rhinitis, atopic dermatitis, and wound healing, comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In yet a further preferred embodiment, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising rheumatic diseases (including but not limited to rheumatoid arthritis, psoriatic arthritis, polymyalgia rheumatica), spondyloarthropathies (including but not limited to ankylosing spondylitis,), connective tissue diseases (including but not limited to systemic lupus erythematosus), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), liver disease (including but not limited to

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- cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis), pulmonary diseases (including but not limited to asthma, chronic obstructive pulmonary disease, adult respiratory distress syndrome), atherosclerosis (eg ischaemic heart disease, myocardial infarction), brain disorders (eg
- 5 multiple sclerosis, demyelinating diseases), psoriasis, transplant rejection, allergic rhinitis, and atopic dermatitis, comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.
- 10 In yet a further preferred embodiment, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising rheumatic diseases (including but not limited to rheumatoid arthritis, psoriatic arthritis, polymyalgia
- 15 rheumatica), spondyloarthropathies (including but not limited to ankylosing spondylitis), connective tissue diseases (including but not limited to systemic lupus erythematosus), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), pulmonary diseases (including but not limited to asthma, chronic obstructive pulmonary disease, adult respiratory distress
- 20 syndrome), atherosclerosis (eg ischaemic heart disease, myocardial infarction), brain disorders (eg multiple sclerosis, demyelinating diseases), psoriasis, and transplant rejection, comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.
- 25 A further aspect of the invention provides for the use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment of a disease or condition as above.

As used herein, the term "effective amount" relates to an amount of compound which,

30 when administered according to a desired dosing regimen, provides the desired MIF cytokine inhibiting or treatment or therapeutic activity, or disease/condition prevention.

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Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. A cytokine or biological activity inhibiting amount is an amount which will at least partially inhibit the cytokine or biological activity of MIF. A therapeutic, or treatment, effective amount is an amount of the compound which, when administered according to a desired dosing regimen, is sufficient to at least partially attain the desired therapeutic effect, or delay the onset of, or inhibit the progression of or halt or partially or fully reverse the onset or progression of a particular disease condition being treated. A prevention effective amount is an amount of compound which when administered according to the desired dosing regimen is sufficient to at least partially prevent or delay the onset of a particular disease or condition. A diagnostic effective amount of compound is an amount sufficient to bind to MIF to enable detection of the MIF-compound complex such that diagnosis of a disease or condition is possible.

Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 μ g to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1 μ g to 1 mg per kg of body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending physician or veterinarian and may depend on the desired level of inhibiting activity, the particular condition being treated, the severity of the condition as well as the general age, health and weight of the subject.

The active ingredient may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition.

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In a further aspect of the invention, there is provided a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof together with a pharmaceutically acceptable carrier, diluent or excipient.

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The formulation of such compositions is well known to those skilled in the art. The composition may contain pharmaceutically acceptable additives such as carriers, diluents or excipients. These include, where appropriate, all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal and antibacterial agents, dermal
10 penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

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The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Compositions include those suitable for oral, rectal, inhalational, nasal, transdermal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intraspinal, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well
20 known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

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Depending on the disease or condition to be treated, it may or may not be desirable for a compound of formula (I) to cross the blood/brain barrier. Thus the compositions for use in the present invention may be formulated to be water or lipid soluble.

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Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount

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of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

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A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg inert diluent, preservative, disintegrant (eg. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose)),
10 surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example,
15 hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Compositions suitable for topical administration in the mouth include lozenges comprising
20 the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

25 The compounds of formula (I) may also be administered intranasally or via inhalation, for example by atomiser, aerosol or nebulizer means.

Compositions suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions,
30 gel, creams, pastes, ointments and the like. Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate,

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polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal devices, such as patches, may also be used to administer the compounds of the invention.

5 Compositions for rectal administration may be presented as a suppository with a suitable carrier base comprising, for example, cocoa butter, gelatin, glycerin or polyethylene glycol.

10 Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides
15 and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example
20 water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-
25 dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the active ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral
30 administration may include such further agents as binders, sweeteners, thickeners, flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or

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time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring.

5 Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents

10 include glyceryl monostearate or glyceryl distearate.

It will be recognised that other therapeutically active agents such as anti-inflammatory (eg steroids such as glucocorticoids) or anti-cancer agents may be used in conjunction with a compound of formula (I). Compounds of formula (I) when administered in conjunction

15 with other therapeutically active agents may exhibit an additive or synergistic effect. These may be administered simultaneously, either as a combined form (ie as a single composition containing the active agents) or as discrete dosages. Alternatively, the other therapeutically active agents may be administered sequentially or separately with the compounds of the invention. Thus, the invention also relates to kits and combinations,

20 comprising a compound of formula (I) and one or more other therapeutically active ingredients for use in the treatment of diseases or conditions described herein.

Without being limiting, examples of agents which could be used in combination with a compound of formula (I) include: glucocorticoids, antirheumatic drugs (including but not

25 limited to methotrexate, leflunomide, sulphasalazine, hydroxychloroquine, gold salts); immunosuppressive drugs (including but not limited to cyclosporin, mycophenyllate mofetil, azathioprine, cyclophosphamide); anti-cytokine therapies (including but not limited to antagonists of, antibodies to, binding proteins for, or soluble receptors for tumor necrosis factor, interleukin 1, interleukin 3, interleukin 5, interleukin 6, interleukin 8,

30 interleukin 12, interleukin 18, interleukin 17, and other pro-inflammatory cytokines as may be found relevant to pathological states); antagonists or inhibitors of mitogen-activated

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protein (MAP) kinases (including but not limited to antagonists or inhibitors of extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK), and the p38 MAP kinases, and other kinases or enzymes or proteins involved in MAP kinase-dependent cell activation); antagonists or inhibitors of the nuclear factor kappa-B (NF- κ B) signal transduction pathway (including but not limited to antagonists or inhibitors of I- κ B-kinase, interleukin receptor activated kinase, and other kinases or enzymes or proteins involved in NF- κ B-dependent cell activation); antibodies, protein therapeutics, or small molecule therapeutics interacting with adhesion molecules and co-stimulatory molecules (including but not limited to therapeutic agents directed against intercellular adhesion molecule-1, CD40, CD40-ligand, CD28, CD4, CD-3, selectins such as P-selectin or E-selectin); bronchodilators such as β -adrenoceptor agonists or anti-cholinergics; antagonists of eicosanoid synthesis pathways such as non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, thromboxane inhibitors, or lipoxygenase inhibitors; antibodies or other agents directed against leukocyte surface antigens (including but not limited to antibodies or other agents directed against CD3, CD4, CD5, CD19, CD20, HLA molecules); agents used for the treatment of inflammatory bowel disease (including but not limited to sulphasalazine, mesalazine, salicylic acid derivatives); anti-cancer drugs (including but not limited to cytotoxic drugs, cytolytic drugs, monoclonal antibodies).

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In another aspect, the invention provides a method of treating or preventing a disease or condition wherein MIF cytokine or biological activity is implicated comprising:

administering to a mammal a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof and a second therapeutic agent.

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In a preferred embodiment of the invention, the second therapeutic agent is a glucocorticoid compound. The mechanism through which MIF antagonises the effects of glucocorticoids has not been fully elucidated. Glucocorticoid effects on inflammation are dependent upon the transactivation of genes which exert inhibitory effects on cell activation, or on the transrepression of genes which exert stimulatory effects on cell

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activation. Transrepression effects are in part mediated via effects on intra-cellular signal transduction pathways such as the nuclear factor κ B (NF- κ B) and mitogen activated protein kinase (MAPK) pathways.

- 5 Without wishing to be bound by theory, it is possible that suppression of activation of signal transduction pathways by a MIF inhibitor may allow a glucocorticoid to be more effective. The ability of glucocorticoids to inhibit the activation of MAPK pathways is uncertain. Glucocorticoids have been variously reported either to suppress, or to be unable to suppress, MAPK activation under various conditions (13-15). Activation of the MAPK
10 pathway known as ERK (extracellular signal regulated kinase, also known as p44/42 MAP kinase), as measured by the phosphorylation of ERK protein detected with a phospho-specific antibody, is increased by stimuli such as interleukin-1 (IL-1) (Figure 3). The ERK pathway is also known to be activated by MIF (16). In experiments using human dermal fibroblasts, the glucocorticoid dexamethasone does not inhibit ERK pathway activation by
15 IL-1. The combination of dexamethasone with a compound that inhibits the cytokine or biological activity of MIF, however, was able to inhibit ERK activation (Figure 3).

Notwithstanding the incomplete understanding of the interacting pathways involved, it is possible that administration of a compound which inhibits the cytokine or biological
20 activity of MIF in combination with a glucocorticoid exerts inhibitory effects on signal transduction pathways that are greater than the effects of the glucocorticoid alone. Where these signal transduction pathways are known to be important in the regulation of cell activation in conditions such as inflammatory diseases, it is likely that this greater effect would permit the use of lower doses of the glucocorticoid in a given patient; that is, the
25 compound which inhibits the cytokine or biological activity of MIF would have a "steroid-sparing" effect.

In another aspect, the present invention provides a method of prophylaxis or treatment of a disease or condition for which treatment with a glucocorticoid is indicated, said method
30 comprising:

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administering to a mammal a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

5 In yet another aspect, the present invention provides a method of treating steroid-resistant diseases comprising:

administering to a mammal a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

10 In a further aspect, the present invention provides a method of enhancing the effect of a glucocorticoid in mammals comprising administering a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof, simultaneously, separately or sequentially with said glucocorticoid.

15 In yet a further aspect, the present invention provides a composition comprising a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof.

20 In a further aspect of the invention there is provided a use of a glucocorticoid in the manufacture of a medicament for administration with a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof, for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

25 In yet a further aspect of the invention there is provided a use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof, in the manufacture of a medicament for administration with a glucocorticoid for the treatment or prophylaxis of a disease or condition for which treatment of a glucocorticoid is indicated.

30 In yet a further aspect of the invention there is provided a use of a glucocorticoid and a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the

manufacture of a medicament for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

Preferably the amount of glucocorticoid used in the methods, uses and compositions of the invention is less than the amount which would be effective in the absence of the compound of formula (I). In the treatment of steroid-resistant diseases or conditions which are not responsive to glucocorticoids, any amount of glucocorticoid which is effective in combination with a compound of formula (I) is considered less than the amount which would be effective in the absence of a compound formula (I). Accordingly, the invention provides a steroid-sparing therapy.

In preferred embodiments of the invention, the glucocorticoid and the compound of formula (I) are used to treat or prevent a disease or condition in a mammal, preferably in a human subject.

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The term "disease or condition for which treatment with a glucocorticoid is indicated" refers to diseases or conditions which are capable of being treated by administration of a glucocorticoid including but not limited to autoimmune diseases, solid or haemopoietic tumours, or chronic or acute inflammatory diseases. Examples of such diseases or conditions include:

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Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration,

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gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg dementia, Alzheimer's disease, multiple sclerosis, demyelinating diseases), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, parturition, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing.

These diseases or conditions may also include steroid-resistant diseases or conditions where treatment with a glucocorticoid is indicated, but where the glucocorticoid is ineffective or is not as effective as expected.

Compounds of formula (I) may be particularly useful in combination with a glucocorticoid, for the treatment of a disease or condition selected from autoimmune diseases, or chronic or acute inflammatory diseases, including rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica)

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spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to myeloma, lymphoma, lung cancer, leukemia, cervical cancer and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (including but not limited to multiple sclerosis, demyelinating diseases), corneal disease, iritis, iridocyclitis, uveitis, sarcoidosis, psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, transplant rejection, allergies, allergic rhinitis, bone diseases (including but not limited to osteoporosis), atopic dermatitis, malarial complications, inflammatory consequences of trauma or ischaemia, and wound healing.

The combination of glucocorticoid and compound of formula (I) may be particularly useful when used in a steroid-sparing manner. The term "steroid-sparing" refers to a combination therapy method that allows a reduction in the amount of glucocorticoid administered while still providing an effective therapy for the disease or condition being treated or prevented.

Steroid-resistant diseases or conditions are diseases or conditions for which treatment with a glucocorticoid is indicated, but where the glucocorticoid is ineffective or is not as effective as expected. This term encompasses diseases or conditions for which the

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effective dose of glucocorticoid results in unacceptable side effects and/or toxicity. Some steroid-resistant diseases or conditions may require a dosage of glucocorticoid so large that they are considered non-responsive and therefore are not able to be successfully treated with glucocorticoids. Some steroid-resistant diseases or conditions may require a large
5 dosage of glucocorticoid to achieve only a small effect on the symptoms of the disease or condition. Furthermore, some patients, diseases or conditions present with symptoms that do not respond to treatment with a glucocorticoid, or may become less sensitive to glucocorticoid treatment over time. Examples of diseases which may commonly exhibit features of steroid-resistance include asthma, chronic obstructive pulmonary disease,
10 rheumatoid arthritis, glomerulonephritis, interstitial nephritis, systemic lupus erythematosus, inflammatory bowel disease and transplant rejection.

Glucocorticoids are a group of steroid hormones, which are used to treat or prevent a wide range of diseases or conditions. Suitable glucocorticoids may be synthetic or naturally
15 occurring and include but are not limited to prednisolone, prednisone, cortisone acetate, beclamethasone, fluticasone, hydrocortisone, dexamethasone, methyl prednisolone, triamcinolone, budesonide and betamethasone. A person skilled in the art would be able to identify other suitable glucocorticoids that may benefit from being used in a combination treatment with a MIF antagonist.

20

In preferred embodiments of the invention, the glucocorticoid used is selected from prednisone, prednisolone, hydrocortisone, fluticasone, beclamethasone, betamethasone, methyl prednisolone, budesonide, triamcinolone, dexamethasone and cortisone. Most preferably, the glucocorticoid is selected from prednisone, prednisolone, methyl
25 prednisolone, fluticasone and beclamethasone. Beclamethasone and fluticasone are particularly preferred for treating asthma. Prednisone, prednisolone and methyl prednisolone are particularly preferred in the treatment of systemic or local inflammatory diseases.

30 The amounts of glucocorticoid and compound of formula (I) are selected such that in combination they provide complete or partial treatment or prophylaxis of a disease or

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condition for which a glucocorticoid is indicated. The amount of compound formula (I) is preferably an amount that will at least partially inhibit the cytokine or biological activity of MIF. The amount of glucocorticoid is preferably less than the amount required in the absence of the compound of formula (I). The amounts of glucocorticoid and compound of formula (I) used in a treatment or therapy are selected such that in combination they at least partially attain the desired therapeutic effect, or delay onset of, or inhibit the progression of, or halt or partially or fully reverse the onset or progression of the disease or condition being treated. The amounts of glucocorticoid and compound of formula (I) used in the prophylaxis of a disease or condition are selected such that in combination they at least partially prevent or delay the onset of the disease or condition. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods.

Suitable doses of a compound of formula (I) may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 μ g to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1 μ g to 1 mg per kg of body weight per dosage.

Suitable dosage amounts of glucocorticoids will depend, in part, on the mode of administration and whether the dosage is being administered in a single, daily or divided dose, or as a continuous infusion. When administered orally, intravenously, intramuscularly, intralesionally or intracavity (eg. intra-articular, intrathecal, intrathoracic), dosages are typically between 1 mg to 1000 mg, preferably 1 mg to 100 mg, more preferably 1 mg to 50 mg or 1 mg to 10 mg per dose. When administered topically or by inhalation as a single, daily or divided dose, dosages are typically 1 ng to 1 μ g, 1 ng to 1 mg or 1 pg to 1 μ g.

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Suitable dosage amounts and dosing regimens can be determined by the attending physician or veterinarian and may depend on the desired level of inhibiting activity, the particular condition being treated, the severity of the condition as well as the general age,
5 health and weight of the subject.

The glucocorticoid and compound of formula (I) may be administered simultaneously or sequentially. The active ingredients may be administered alone but are preferably administered as a pharmaceutically acceptable composition or separate pharmaceutically
10 acceptable compositions.

The formulation of such compositions is well known to those skilled in the art and are described above in relation to compounds of formula (I). The composition or compositions may contain pharmaceutically acceptable additives such as carriers, diluents
15 or excipients. These include, where appropriate, all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

20

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the glucocorticoids and/or compound of formula (I) which inhibit the cytokine or biological activity of MIF.

25 In one preferred aspect of the invention, the compounds of formula (I) may be administered together with, simultaneously or sequentially, glucocorticoids. In such a therapy, the amount of glucocorticoid required may be significantly reduced.

The compounds of formula (I), either as the only active agent or together with another
30 active agent, e.g. a glucocorticoid, may also be presented for use in veterinary

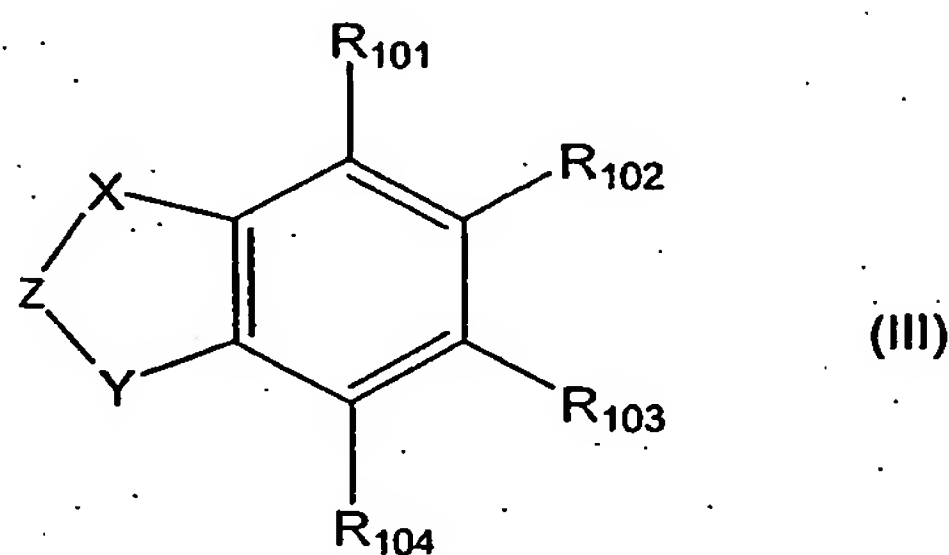
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compositions. These may be prepared by any suitable means known in the art. Examples of such compositions include those adapted for:

- (a) oral administration, external application (eg drenches including aqueous and non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pellets for admixture with feedstuffs, pastes for application to the tongue;
- (b) parenteral administration, eg subcutaneous, intramuscular or intravenous injection as a sterile solution or suspension; and
- (c) topical application eg creams, ointments, gels, lotions, etc.

By virtue of their ability to bind to or antagonize MIF, compounds of formula (I) or salts or derivatives thereof may be used as laboratory or diagnostic or *in vivo* imaging reagents. Typically, for such use the compounds would be labelled in some way, for example, radio isotope, fluorescence or colorimetric labelling, or be chelator conjugated. In particular, compounds of formula (I) could be used as part of an assay system for MIF or as controls in screens for identifying other inhibitors. Those skilled in the art are familiar with such screens and could readily establish such screens using compounds of formula (I). Those skilled in the art will also be familiar with the use of chelate conjugated molecules for *in vivo* diagnostic imaging.

In a further aspect of the invention there is provided a compound of formula (III) or a pharmaceutically acceptable salt or prodrug thereof:



wherein

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X is -O-, -NH- or -CH₂-;

Y is -NH-, -O-, -S- or -CH₂-;

5 Z is -C(O)-, -C(S)- or -S(O)-;

R₁₀₁ is selected from hydrogen, C₁₋₃alkyl, OH, SH, NH₂, NHC₁₋₃alkyl, F, Cl or Br;

10 R₁₀₂ is selected from C₁₋₂₀alkyl, C₂₋₂₀alkenyl, CO₂H, F, Cl, Br, CO₂R₁₀₅, (CH₂)_wR₁₀₆, C(O)N(R₁₀₇)₂, C(=N)NHC₁₋₆alkyl, SO₂C₁₋₆alkyl, C(O)[NHCH(R₁₀₈)C(O)]_q-OR₁₀₉, NH₂, C(O)sugar, CONH(CH₂)_naryl, NHC(O)(CH₂)_nSheterocyclyl, C(O)SC₁₋₆alkyl, C(O)(CH₂)_nCO₂H, SO₂OC₁₋₁₀alkyl, SO₂NHC₁₋₁₀alkyl;

15 R₁₀₃ is selected from hydrogen, F, Cl, Br, C₁₋₆alkyl, -(CH₂)_nNH₂, -(CH₂)_nNO₂, -(CH₂)_n-OH, -(CH₂)_n-CF₃, -(CH₂)_nC(O)C₁₋₃alkyl or -(CH₂)_n-SH;

R₁₀₄ is selected from hydrogen, methyl, ethyl, CH₂C(R₁₁₀)₃, C(R₁₁₀)₃, -CH₂=CH₂, fluoro, chloro or bromo;

20 R₁₀₅ is selected from hydrogen, C₁₋₂₀alkyl, C₂₋₂₀alkenyl or (CH₂)_iOC₁₋₃alkyl;

R₁₀₆ is selected from SH, SC₁₋₆alkyl, OH, OC₁₋₆alkyl, sugar, CO₂H, NH₂, heterocyclyl or aryl;

25 Each R₁₀₇ is independently selected from hydrogen, C₁₋₂₀alkyl, C₂₋₂₀alkenyl, (CH₂)_iaryl and (CH₂)_iheterocyclyl;

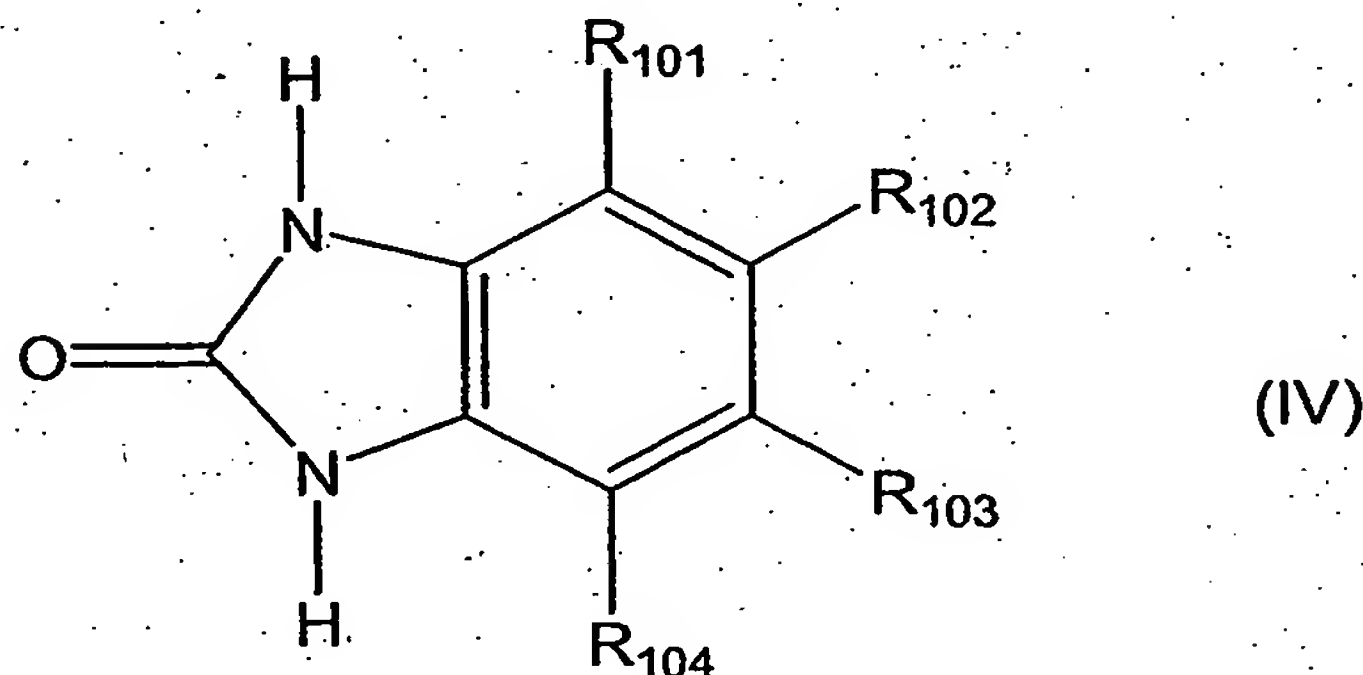
R₁₀₈ is the characterising group of an amino acid;

30 R₁₀₉ is hydrogen, C₁₋₃alkyl;

Each R_{110} is independently selected from hydrogen and halo; and

n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6; t is an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

Preferred compounds of formula (III) are benzimidazole compounds having formula (IV):



wherein

R_{101} is selected from hydrogen, CH_3 , OH, SH, NH_2 , $NHCH_3$, F, Cl or Br;

R_{102} is selected from C_{1-20} alkyl, C_{2-20} alkenyl, CO_2H , F, Cl, Br, CO_2R_{105} , $(CH_2)_wR_{106}$, $C(O)N(R_{107})_2$, $C(=N)NHC_{1-6}$ alkyl, SO_2C_{1-6} alkyl, $C(O)[NHCH(R_{108})C(O)]_q-OR_{109}$, NH_2 , $C(O)sugar$, $CONH(CH_2)_naryl$, $NHC(O)(CH_2)_nSheterocyclyl$, $C(O)SC_{1-6}$ alkyl, $C(O)(CH_2)_nCO_2H$, SO_2OC_{1-10} alkyl, SO_2NHC_{1-10} alkyl;

R_{103} is selected from hydrogen, F, Cl, Br, C_{1-6} alkyl, $(CH_2)_nNH_2$, $-(CH_2)_nNO_2$, $-(CH_2)_n-OH$, $-(CH_2)_n-CF_3$, $CH_2C(O)CH_3$ or $-(CH_2)_n-SH$;

R_{104} is selected from hydrogen, methyl, ethyl, CH_2CF_3 , $-CH_2=CH_2$ fluoro, chloro or bromo;

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R₁₀₅ is selected from hydrogen, C₁₋₁₀alkyl, C₂₋₁₀alkenyl, (CH₂)_tOC₁₋₃alkyl;

5 R₁₀₆ is selected from SH, SC₁₋₆alkyl, OH, OC₁₋₆alkyl, sugar, CO₂H, NH₂, heterocyclyl or aryl;

Each R₁₀₇ is independently selected from hydrogen, C₁₋₁₀alkyl, C₂₋₁₀alkenyl, (CH₂)_taryl and (CH₂)_theterocyclyl;

10 R₁₀₈ is the characterising group of an amino acid;

R₁₀₉ is hydrogen, C₁₋₃alkyl;

15 Each R₁₁₀ is independently selected from hydrogen and halo; and

n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6, t is an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

20 Preferably the compounds of formula (III) or formula (IV) are those in which at least one or more of the following definitions apply:

R₁₀₁ is hydrogen, F, Cl or Br;

25 R₁₀₂ is C₁₋₂₀alkyl, halogen, NH₂, CO₂H, CO₂C₁₋₁₀alkyl, C(O)sugar, CO₂(CH₂)_nOC₁₋₆alkyl, CONHC₁₋₁₀alkyl, CONH(CH₂)_naryl, CO[NHCH(R₁₀₇)CO]-OH, CO[NHCH(R₁₀₇)CO]OC₁₋₃alkyl, NHC(O)(CH₂)_nSheterocyclyl, C(O)SC₁₋₆alkyl, C(O)(CH₂)_nCO₂H, SO₂OC₁₋₁₀alkyl, SO₂NHC₁₋₁₀alkyl or C(=NH)NHC₁₋₆alkyl;

30 R₁₀₃ is hydrogen, halogen, C₁₋₃alkyl, (CH₂)_nNH₂, (CH₂)_nNO₂, (CH₂)_nOH or (CH₂)_nCF₃;

R₁₀₄ is hydrogen, F, Cl or Br;

R₁₀₈ is the characterising group of an amino acid, preferably the characterising group from serine (CH₂OH) or phenylalanine (CH₂Ph);

5

n is 0 or an integer from 1 to 3; and

wherein each alkyl or aryl group is optionally substituted, preferably with one or more OH, carboxylic acid or halo.

10

Preferred compounds of formula (III) include:

15

benzimidazol-2-one-5-n-pentanoate,

5[2(1-oxy-2-hydroxyethyl)ethyl]benzimidazol-2-one-5-carboxylate,

benzimidazol-2-one-5-methanoate,

benzimidazol-2-one-5-ethanoate,

pentyl-benzimidazole-2-thioxo-5-carboxylate,

3,4,5-tris(acetyloxy)-6-[(acetyloxy)methyl]tetrahydro-2H-pyran-2-yl-

benzimidazole-2-one-5-carboxylate,

20

5-bromo-6-methylbenzimidazol-2-one,

5-hydroxy-6-methylbenzimidazol-2-one,

5-dodecanylbzenzimidazol-2-one,

4,5,7-tribromo-6-methylbenzimidazol-2-one,

4,5,6,7-tetrabromobenzimidazol-2-one,

25

5-methyl-6-nitrobenzimidazol-2-one,

5-amino-6-methylbenzimidazol-2-one,

N-(6-methylbenzimidazol-5-yl)-2-pyrimidin-2-yl-sulfanyl-acetamide

pentyl-benzimidazol-2-one-5-carbothioate

5-(benzimidazol-2(3H)-one-6-yl)-5-oxopentanoic acid

30

2(3H)-benzimidazolone-5-sulfonic acid pentyl ester,

2(3H)-benzimidazolone-5-sulfonic acid pentyl amide,

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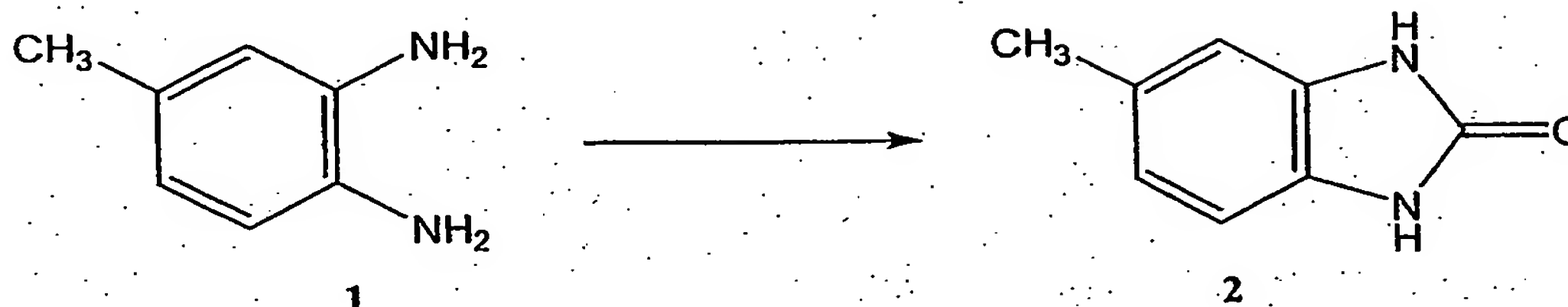
N-butyl-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboximidamide
5-heptanoylbenzofuran-2(3H)-one,
methyl 3-hydroxy-2-{[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}propanoate,
5 3-hydroxy-2-{[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}propanoic acid,
methyl 2-{[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenyl propanoate,
2-{[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenyl
10 propanoic acid, and
N-(3,4-dihydroxyphenethyl)-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxamide.

Unless the context indicates otherwise, reference to any prior art in this specification is not,
15 and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood
20 that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

25 The invention will now be described with reference to the following examples which are included for the purpose of illustration only and are not intended to limit the generality of the invention hereinbefore described.

EXAMPLES

Synthesis of compounds of Formula (I).Example 1:5 5-Methylbenzimidazol-2-one (2)

This was prepared as described by *Harvey et al* (12).

- 10 A solution of urea (6.00 g, 0.1000 mol) and 3,4-diaminotoluene (1) (12.20 g, 0.0999 mol) in pentan-1-ol (40 mL) was vigorously stirred and heated to reflux under a nitrogen atmosphere. After 2 hours the heating was discontinued and on cooling to room temperature a pink solid settled out. This was filtered off and recrystallised from boiling ethanol (17.98 g. in 400 mL) to give 2 crops of 5-methylbenzimidazol-2-one (2) as a pink powder. The total mass was recovered was 8.21 g (56 % yield);
- 15

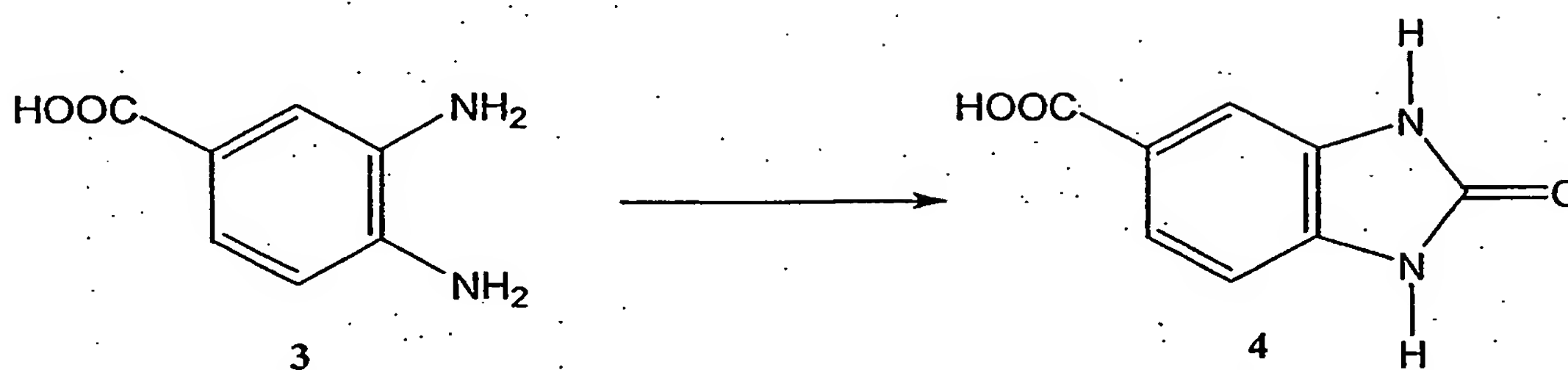
R_f : 0.40 (9:1 CHCl_3 :MeOH),

mp: 300-302 °C, lit.³ mp: 297-300 °C;

^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$): δ 2.12 (s, 3 H, CH_3), 6.63-6.70 (m, 3H, ArH);

- 20 LRESI mass spectrum: m/z 149 (100%, MH^+).

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Example 2:Benzimidazol-2-one-5-carboxylic acid (4)

5

The method described by *Harvey et al* (12) and used in Example 1 for the preparation of 5-methylbenzimidazol-2-one (2) was used except this preparation started with 3,4-diaminobenzoic acid (3).

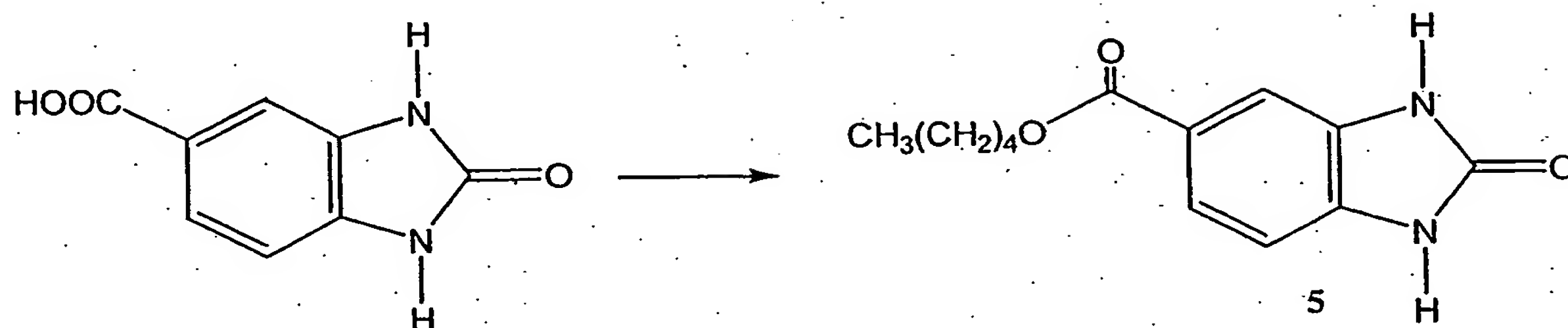
- 10 Urea (1.20 g, 0.0200 mol) and 3,4-diaminobenzoic acid (3) (3.04 g, 0.0200 mol) in pentan-1-ol (10 mL) was vigorously stirred and heated to reflux under a nitrogen atmosphere. The heating was discontinued after 4 hours and on cooling to room temperature, water (30 mL) was added. The pH was adjusted to 1 with conc. HCl. The resultant dark solid was filtered off, washed with further water (2 x 20 mL) and dried to give 3.00 g (84% yield) of
- 15 benzimidazol-2-one-5-carboxylic acid (4) as a black powder;

R_f : 0.09 (9:1 CHCl_3 :MeOH), 0.20 (4:1 CHCl_3 :MeOH),

$^1\text{H NMR}$ (d_6 -DMSO): δ 6.98 (d, 1 H, $J_{7,6}$ 8.1 Hz, H-7), 7.45(d, 1 H, $J_{4,6}$ 1.2 Hz, H-4), 7.60 (dd, 1 H, H-6), 10.78 (bs, 1 H, NH), 10.94 (bs, 1 H, NH);

- 20 LRESI negative ion mass spectrum: m/z 177 (100%, M-H^-).

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Example 3:Benzimidazol-2-one-5-n-pentanoate (5)

5

Benzimidazol-2-one-5-carboxylic acid (4) (250 mg, 0.9070 mmol) and Dowex 50W-X8(H⁺) resin (250 mg) were suspended in pentan-1-ol (40 mL) and the mixture heated to reflux for 42 hours. The solid was filtered off and washed with methanol (3 x 20 mL) and the combined filtrates evaporated to dryness to give benzimidazol-2-one-5-*n*-pentanoate (5) (310 mg, 43% yield) as an off-white powder;

10

R_f: 0.63 (4:1 CHCl₃:MeOH),

mp: 227-228°C,

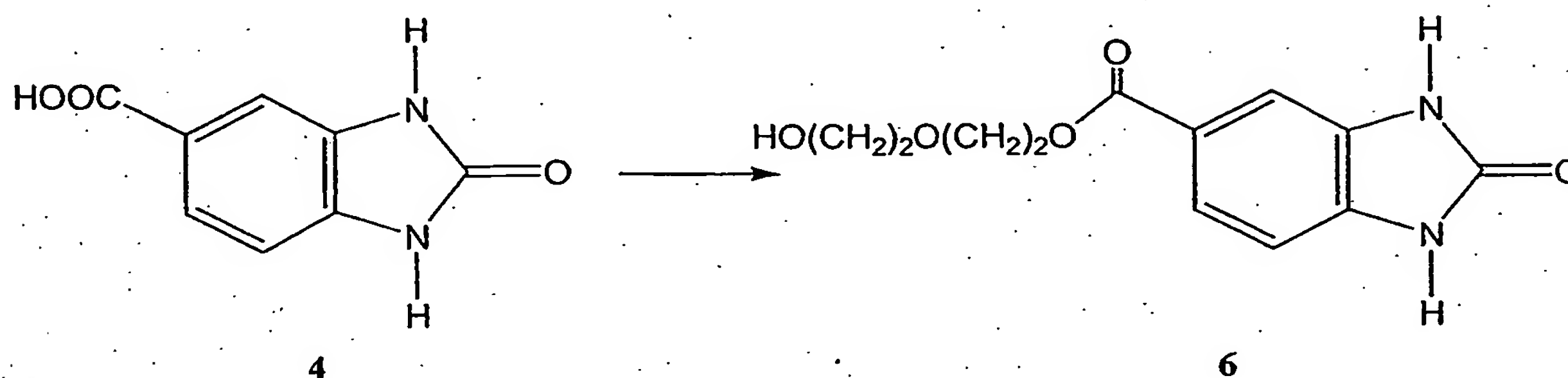
¹H NMR (CDCl₃/CD₃OD): δ 0.88-0.92 (pseudo t, 3 H, CH₃), 1.33-1.43 (m, 4 H, 2 x CH₂),
 15 1.70-1.79 (m, 2 H, CH₂), 4.25-4.29 (pseudo t, 2 H, CH₂), 7.04 (d, 1 H, J_{7,6} 8.4 Hz, H-7),
 7.44 (bs, 1 H, NH), 7.55 (bs, 1 H, NH), 7.66 (bs, 1 H, H-4), 7.75 (dd, 1 H, J_{6,5} 1.5 Hz, H-6);

LRESI negative ion mass spectrum: *m/z* 247 (100%, [M-H]⁻);

HRESI positive ion mass spectrum: C₁₃H₁₇N₂O₃ calculated 249.12391,

20 C₁₃H₁₆N₂O₃ calculated C, 62.97; H, 6.50; N, 11.29, found C, 63.1, H, 6.54, N, 11.05.

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Example 4:5 [2(1-oxy-2-hydroxyethyl)ethyl] benzimidazol-2-one-5-carboxylate (6)

5 Benzimidazol-2-one-5-carboxylic acid (4) (300 mg, 1.6853 mmol) and Dowex 50W-X8(H⁺) resin (300 mg) were suspended in diethylene glycol (50 mL) and the mixture heated to reflux for 44 hours. The solid was filtered off and washed with methanol (3 x 20 mL) and the combined filtrates reduced in volume (approx 2 mL) with vacuum distillation.

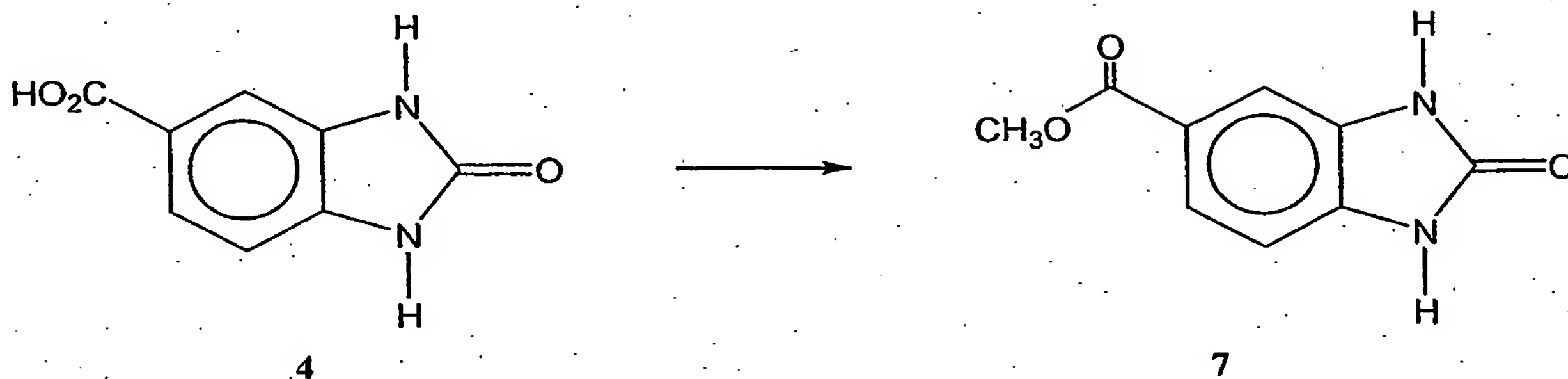
10 This residue was column chromatographed (SiO₂, isocratically with 4:1 CHCl₃:MeOH) to give with evaporated to dryness to give 5 [2(1-oxy-2-hydroxyethyl)ethyl] benzimidazol-2-one-5-carboxylate (6) (310 mg, 43% yield) as an off-white powder;

R_f: 0.63 (4:1 CHCl₃:MeOH),

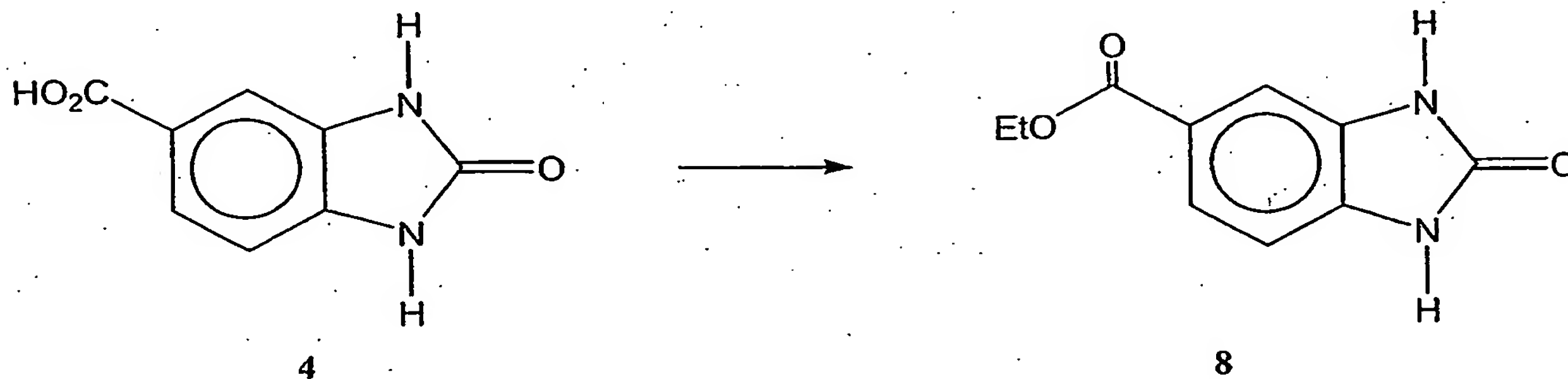
15 mp: 227-228°C,

¹H NMR (CDCl₃/CD₃OD): δ 0.88-0.92 (pseudo t, 3 H, CH₃), 1.33-1.43 (m, 4 H, 2 x CH₂), 1.70-1.79 (m, 2 H, CH₂), 4.25-4.29 (pseudo t, 2 H, CH₂), 7.04 (d, 1 H, J_{7,6} 8.4 Hz, H-7), 7.44 (bs, 1 H, NH), 7.55 (bs, 1 H, NH), 7.66 (bs, 1 H, H-4), 7.75 (dd, 1 H, J_{6,5} 1.5 Hz, H-6);

20 LRESI negative ion mass spectrum: m/z 247 (100%, [M-H]⁻);

Example 5:benzimidazol-2-one-5-methanoate

- 5 Benzimidazol-2-one-5-carboxylic acid (4) (100 mg; 0.56 mmol) and DCC (100 mg) were suspended in methanol and the mixture heated to reflux for 42 hours. The solid was filtered off and washed with methanol (3 x 3 mL) and the combined filtrates evaporated to dryness to give benzimidazol-2-one-5-methanoate (7) in 61% yield.
- 10 ¹HMR(d₆-DMSO): δ 3.80 (s, 3H, CO₂CH₃), 6.70 (d, 1H, *J*_{ortho} 8.1 Hz, aromatic), 7.46 (bs, 1H, 4-H aromatic), 7.61 (d, 1H, *J*_{ortho} 8.1 Hz, aromatic), 10.82 (bs, 1H, NH) and 10.99 (bs, 1H, NH).
- Negative ion mass spectrum: *m/z* 191 (40%, M-1⁺).

15 Example 6:benzimidazol-2-one-5-ethanoate

- 20 Benzimidazol-2-one-5-carboxylic acid (4) (100 mg) and conc. H₂SO₄ (0.25 mL) were suspended in ethanol and the mixture heated to reflux for 20 hours. The solid was filtered

- 70 -

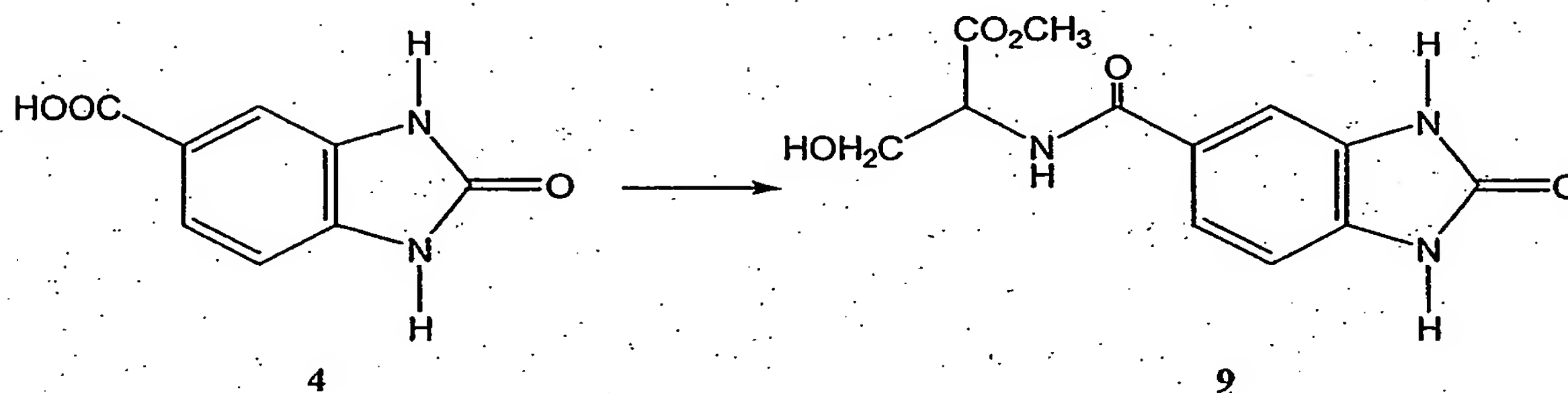
off and washed with ethanol (50 mL) and the combined filtrates evaporated to dryness to give benzimidazol-2-one-5-ethanoate (8) in 69% yield.

¹HMR(d₆-DMSO): δ 1.20 (t, 3H, J=6.9 Hz, CH₃-), 4.26 (q, 2H, J=6.9 Hz, OCH₂-), 7.00 (d, 1H, J_{ortho} 8.1 Hz, H-7 aromatic), 7.46 (d, 1H, J_{meta} 1.5 Hz, 4-H aromatic), 7.62 (dd, 1H, J_{ortho} 8.2 Hz and J_{meta} 1.5 Hz, 6-H aromatic) and 10.88 (bs, 2H, NH).

Negative ion mass spectrum: M/Z 205 (100%, M-1⁺).

Example 7:

10 methyl 3-hydroxy-2-{[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino} propanoate (9)



15 A suspension of benzimidazol-2-one-5-carboxylic acid (4) (356 mg, 2.00 mmol) and L-serine-methylester hydrochloride (311 mg, 2.00 mmol) in sieve dried DMF (6 mL) was cooled in an ice-bath. Then added, in the following sequence, were 1-hydroxybenzotriazole monohydrate (612 mg, 4.00 mmol), diisopropylethylamine (0.696 mL, 517 mg, 4.00 mmol, Hunnig's Base) and 1,3-dicyclohexylcarbodiimide (412 mg, 2.00 mmol). The reaction was allowed to equilibrate to room temperature and left to stir for 41 hours.

The white solid dicyclohexylurea was then filtered off and washed with further DMF (5 mL). The combined filtrates were then vacuum distilled to give a black oil (2.09 g). This was triturated with chloroform (20 mL) over ice to give a dark solid that was filtered off. A sample (250 mg) was made up as a DMF bolus and this column chromatographed (SiO₂,

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isocratically eluted with 4:1 CHCl₃:MeOH) to give 208 mg of the benzimidazol-2-one-5-Serine-amido-coupled adduct (9) as a pale brown powder;

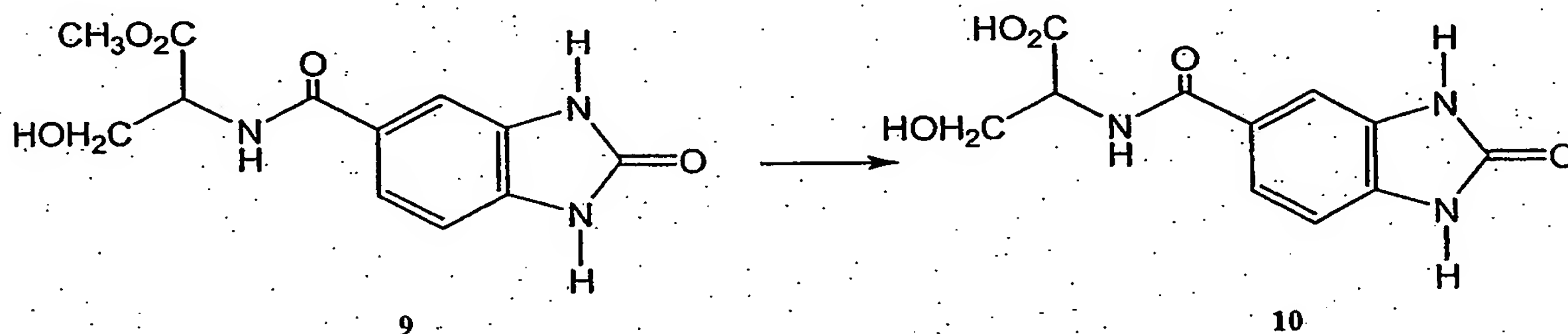
R_f: 0.41 (4:1 CHCl₃:MeOH),

¹H NMR (d₆-DMSO): δ 3.63 (s, 3 H, CO₂CH₃), 3.75-3.79 (pseudo t, 2 H, CH₂), 4.48-4.54 (m, 1 H, CH), 5.00 (t, 1 H, OH), 6.97 (d, 1 H, J_{7,6} 8.1 Hz, H-7), 7.47 (bs, 1 H, H-4), 7.54 (dd, 1 H, J_{6,5} 1.5 Hz, H-6), 8.35 (d, 1 H, J_{NH} 7.5 Hz, NH), 10.83 (bs, 2 H, 2 x NH);

LRESI mass spectrum: m/z 280 (100%, MH⁺).

Example 8:

3-hydroxy-2-[[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino]propanoic acid (10)



The methyl ester adduct benzimidazol-2-one-5-carboxy-(L-serine)-amide (9) (100 mg, 0.3584 mmol) was suspended in methanol (10 mL) and on addition of 1M aq NaOH (0.68 mL, 0.680 mmol) readily dissolved. The disappearance of starting material was monitored with TLC and complete after stirring overnight at room temperature. The volume was increased by addition of further methanol (30 mL) and the pH carefully adjusted from 10 to 5 by the addition of Dowex 50W-X8(H⁺) resin. The resin was rapidly filtered off and washed with further methanol (4 x 20 mL) and the combined filtrates rotary evaporated to dryness to give benzimidazol-2-one-5-carboxy-(L-serine)-amide (10) (91 mg, 96% yield) as a white powder;

R_f: ≤0.04 (4:1 CHCl₃:MeOH),

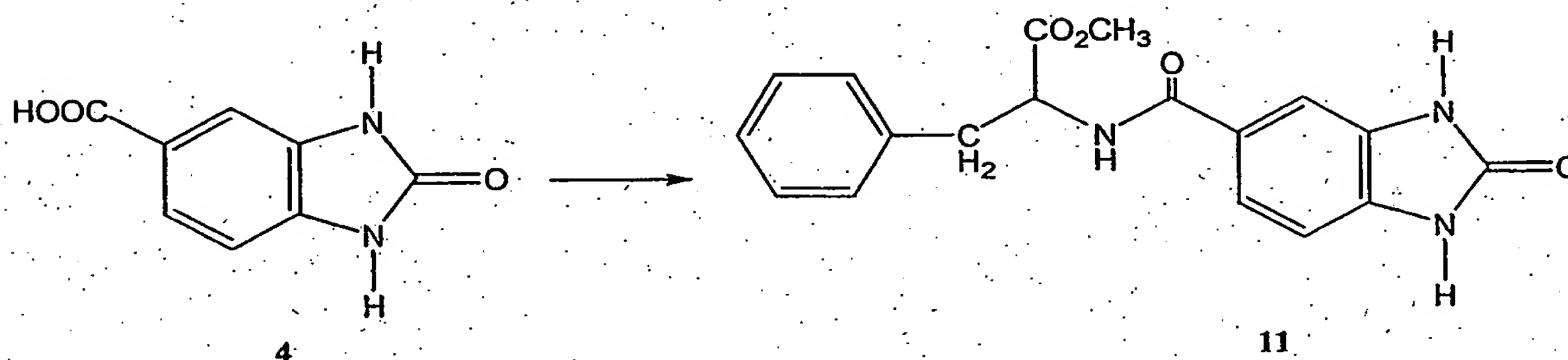
- 72 -

¹H NMR (d₆-DMSO): δ 3.74-3.80(m, 2 H, CH₂), 4.38-4.45(m, 1 H, CH), 6.97(d, 1 H, *J*_{7,6} 8.1 Hz, H-7), 7.46 (bs, 1 H, H-4), 7.53 (dd, 1 H, *J*_{6,5} 1.5 Hz, H-6), 8.16 (d, 1 H, *J*_{NH} 7.5 Hz, NH);

LRESI mass spectrum: positive ion *m/z* 266 (100%, MH⁺), negative ion *m/z* 264 (100%, [M-H]⁻).

Example 9:

methyl 2-{[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenylpropanoate (11)



A suspension of benzimidazol-2-one-5-carboxylic acid (4) (356 mg, 2.00 mmol) and L-phenylalanine-methylester hydrochloride (431 mg, 2.00 mmol) in sieve dried DMF (6 mL) was cooled in an ice-bath. Then added, in the following sequence, were 1-hydroxybenzotriazole monohydrate (612 mg, 4.00 mmol), diisopropylethylamine (0.696 mL, 517 mg, 4.00 mmol, Hunnig's Base) and 1,3-dicyclohexylcarbodiimide (412 mg, 2.00 mmol). The reaction was allowed to equilibrate to room temperature and left to stir for 44 hours.

The white solid dicyclohexylurea was then filtered off and washed with further DMF (5 mL). The combined filtrates were then vacuum distilled to give a black oil (2.00 g). This was column chromatographed (SiO₂, isocratically eluted with 6:1 CHCl₃:MeOH) to give as main product 1.15 g of a brown solid. 150 mg was taken and this then rechromatographed (SiO₂, isocratically eluted with 9:1 CHCl₃:MeOH) to give 81 mg (equivalent to 90% overall yield) of the benzimidazol-2-one-5-phenylalanine-amido-coupled adduct (11) as an off white powder;

- 73 -

R_f : 0.38 (9:1 CHCl_3 :MeOH),

mp: 220-221°C;

^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$): δ 3.06-3.13 (m, 2 H, CH_2), 3.63 (s, 3 H, CO_2CH_3),

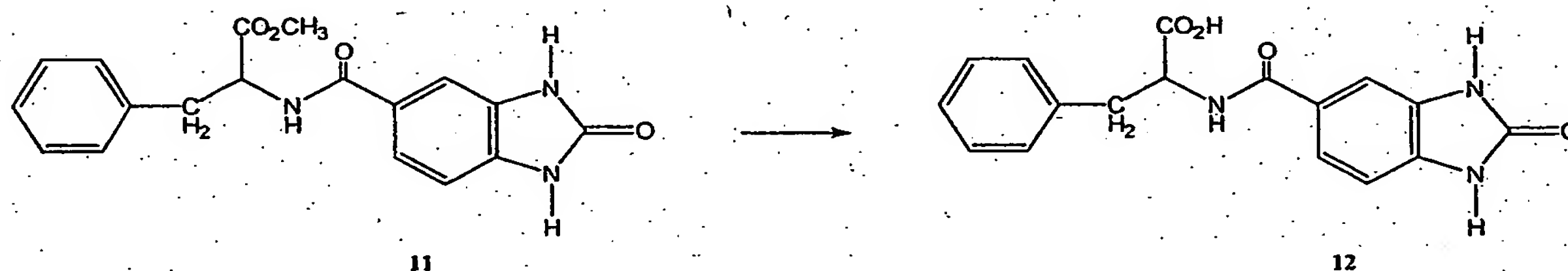
5 4.86 (pseudo t, 1 H, CH), 6.90 (bd, 1 H, J 8.1 Hz), 7.03-7.05 (m, 2 H),

7.10-7.18 (m, 3 H), 7.28-7.31 (m, 2 H, J 8.1 Hz, J 1.5 Hz);

LRESI mass spectrum: m/z 340 (41%, MH^+), 225 (100%).

Example 10:

10 2-{\[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenyl propanoic acid (12)



15 1M aq NaOH (0.75 mL, 0.75 mmol) was added to the methyl ester adduct of benzimidazol-2-one-5-carboxy-(L-phenylalanine)-amide (11) (65 mg, 0.1917 mmol) dissolved in methanol (7.5 mL) and this stirred overnight at room temperature. The volume was increased then increased to 50 mL by the addition of further methanol and the pH then carefully adjusted from 10 to 5 by the addition of Dowex 50W-X8(H^+) resin. The

20 resin was rapidly filtered off, washed with further methanol (4 x 20 mL) and the combined filtrates rotary evaporated to dryness to give an oil. This was taken up in hot ethanol and on cooling a white solid settled out from solution. This was filtered off and dried to give benzimidazol-2-one-5-carbox-(L-phenylalanine)-amide (12) (60 mg, 97% yield) as a white powder;

25

R_f : ≤ 0.03 (9:1 CHCl_3 :MeOH),

mp: 222-223 °C,

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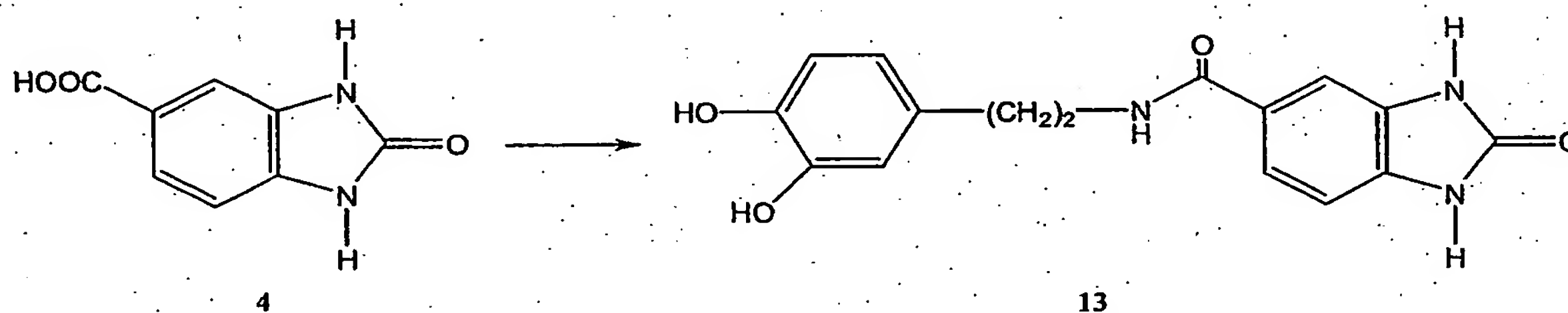
^1H NMR (CD_3OD): δ 3.13 (dd, 1 H, J_{geminal} 13.5 Hz, J 7.8 Hz, benzyl- CH_2), 3.34 (dd, 1 H, benzyl- CH_2), 4.76 (pseudo t, 1 H, J 7.5 Hz, CH), 7.03 (bd, 1 H, J 8.1 Hz), 7.12-7.27 (m, 5 H), 7.43-7.47 (m, 2 H, J 8.4 Hz, J 1.5 Hz);

LRESI mass spectrum: m/z 326 (100%, MH^+).

5

Example 11:

N-(3,4-dihydroxyphenethyl)-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxamide (13)



10

A suspension of benzimidazol-2-one-5-carboxylic acid (4) (356 mg, 2.00 mmol) and 3,4-dihydroxyphenylethylamine hydrochloride (379 mg, 2.00 mmol) in sieve dried DMF (6 mL) was cooled in an ice-bath. Then added, in the following sequence, were 1-hydroxybenzotriazole monohydrate (612 mg, 4.00 mmol), diisopropylethylamine (0.696 mL, 517 mg, 4.00 mmol, Hunnig's Base) and 1,3-dicyclohexylcarbodiimide (412 mg, 2.00 mmol). The reaction was allowed to equilibrate to room temperature and left to stir for 44 hours.

The white solid dicyclohexylurea was then filtered off and washed with further DMF (2 x 5 mL). The combined filtrates were then vacuum distilled to give a dark brown oil (2.39 g). This was taken up in methanol (5 mL) and on addition of chloroform (10 mL) a dark solid settled out of solution. This was filtered off, dissolved in a minimum amount of DMF and then subject to column chromatography (SiO_2 , isocratically eluted with 4:1 CHCl_3 :MeOH) to give 604 mg (96 % yield) of benzimidazol-2-one-5-dopamine-amido-coupled adduct (13) as a pale brown powder. A sample was triturated with cold 4:1 CHCl_3 :MeOH, filtered and dried to give material for spectroscopic and bioassay analysis.

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R_f : 0.38 (9:1 CHCl_3 :MeOH),

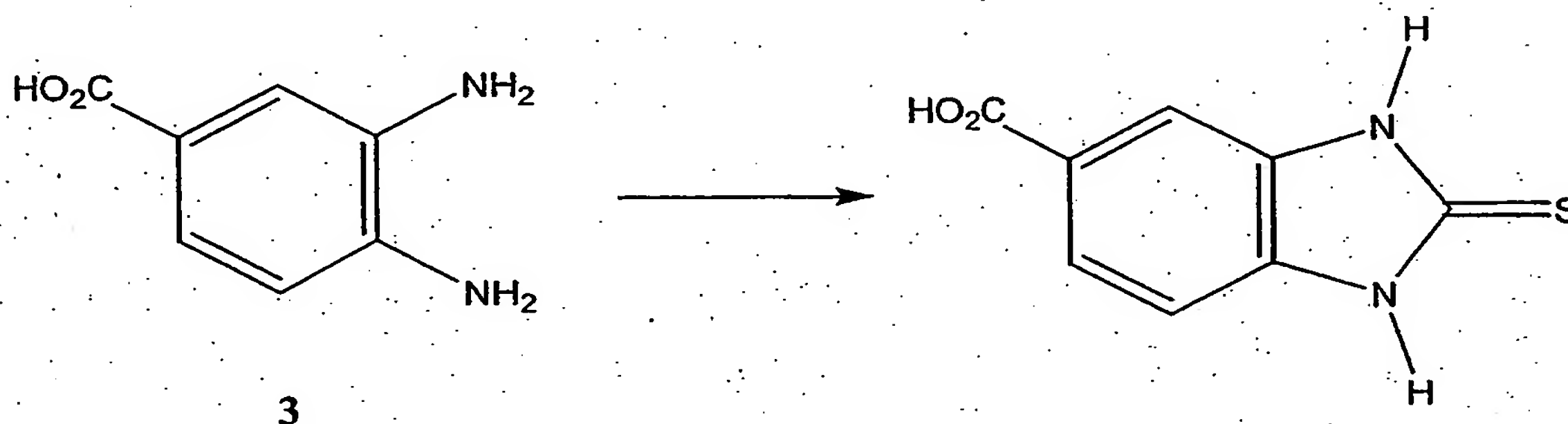
mp: >250 °C, darkens without melting;

^1H NMR (d_6 DMSO): δ 2.49 (t, 2 H, J 1.8 Hz, CH_2), 2.62 (t, 2 H, J 78 Hz, CH_2), 6.45 (dd, 1 H, $J_{6,5}$ 8.1, $J_{6,2}$ 1.8 Hz, H-6') 6.60-6.64 (m, 2 H, H-2', H-5'), 6.94 (d, 1 H, $J_{7,6}$ 8.4 Hz, H-7), 7.42 (bs, 1 H, H-4), 7.47 (dd, 1 H, $J_{6,4}$ 1.5 Hz, H-4), 8.33 (bt, 1 H, J 5.4 Hz, amide NH), 8.63 (bs, 1 H, hetero NH), 8.73 (bs, 1 H, hetero NH);

LRESI mass spectrum: m/z , negative ion 312 (63%, $[\text{M}-\text{H}]^-$), (249, 39%), (134, 100%); positive ion 314 (37%, MH^+), (211, 69%), (130, 100%).

10 Example 12:

benzimidazol-2-thio-5-carboxylic acid (14)

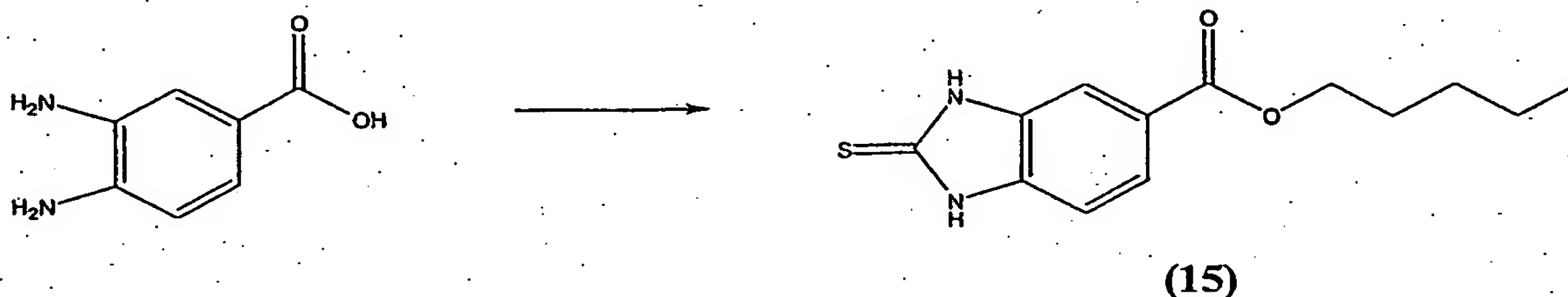


- 15 The method described by Harvey *et al.* (12) and used in Example 2 for the preparation of benzimidazol-2-one-5-carboxylic acid (4) was used except this preparation used thiourea instead of urea.

Thiourea (1.52 g, 2 mmol) and 3,4-diaminobenzoic acid (3) (3.04 g, 2 mmol) in pentan-1-
 20 ol (14 ml) was stirred vigorously and heated to reflux under nitrogen atmosphere. Heating was discontinued after 5 hours, and stirring continued for 12 hours. Water was added and the pH adjusted to 1. The solvents were removed and the black solid was triturated with cold water (10 ml) and filtered to give 1.94 g of a black powder.

LRESI negative ion in mass spectrum: m/z 193 ($\text{M}-\text{H}^-$).

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Example 13:Pentyl -benzimidazole-2-thioxo-5-carboxylate (15)

To a mixture of the diamine (500 mg, 3.28 mmol) in anhydrous 1-pentanol (50 mL) was added thiourea (300 mg, 3.94 mmol). The mixture was refluxed for 6 hrs, and then cooled before filtering to furnish a black solid (471 mg, 74 %). The solid was washed with 1M HCl and water. The dried solid (200 mg) was then suspended in anhydrous 1-pentanol (25 ml), to which was added Dowex H⁺ resin (200 mg). The mixture was refluxed for 42 hrs. The reaction mixture was cooled and filtered, and the filtrate concentrated to furnish the title compound as a black gum (193 mg, 52% overall yeild).

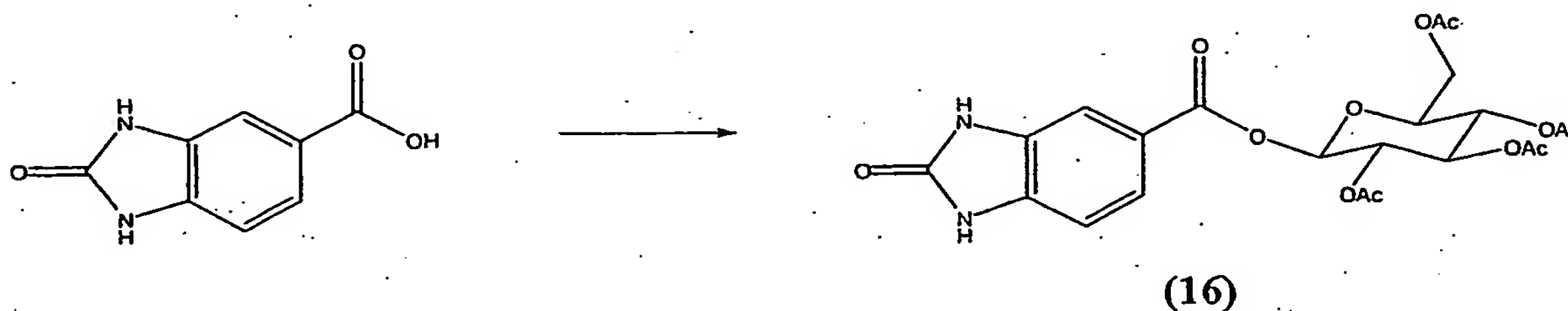
15 ¹H NMR (CDCl₃): δ 8.41 (s, 1H, ArH), 8.03 (d, 1H, ArH), 7.82 (d, 1H, ArH), 4.30 (t, 2H, -OCH₂CH₂CH₂CH₂CH₃), 1.75 (m, 1H, , -OCH₂CH₂CH₂CH₂CH₃), 1.38 (m, 4H, , -OCH₂CH₂CH₂CH₂CH₃), 0.89 (m, 3H, , -OCH₂CH₂CH₂CH₂CH₃);

LRMS (ESI): *m/z* 265 [M+H⁺];

C₁₃H₁₆N₂O₂S: 264.34

20 Example 14:

3,4,5-tris(acetyloxy)-6-[(acetyloxy)methyl]tetrahydro-2H-pyran-2-yl-benzimidazole-2-one-5-carboxylate (16)



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To a solution of the acid (128 mg, 0.72 mmol) in anhydrous pyridine (20 mL) was added Ag_2CO_3 (265 mg, 0.96 mmol), and molecular sieves (4Å, 2.7 g). The mixture was stirred under an atmosphere of nitrogen at room temperature for 30 minutes. Tetra-*O*-acetyl-glucopyranosyl bromide (100 mg, 0.24 mmol) was added to the reaction mixture, and stirred overnight at room temperature. The mixture was then filtered through celite and the filtrate concentrated to an amber gum. The gum was dissolved in chloroform (5 mL), and triturated with ether to furnish the title compound as a white solid (19 mg, 15%).

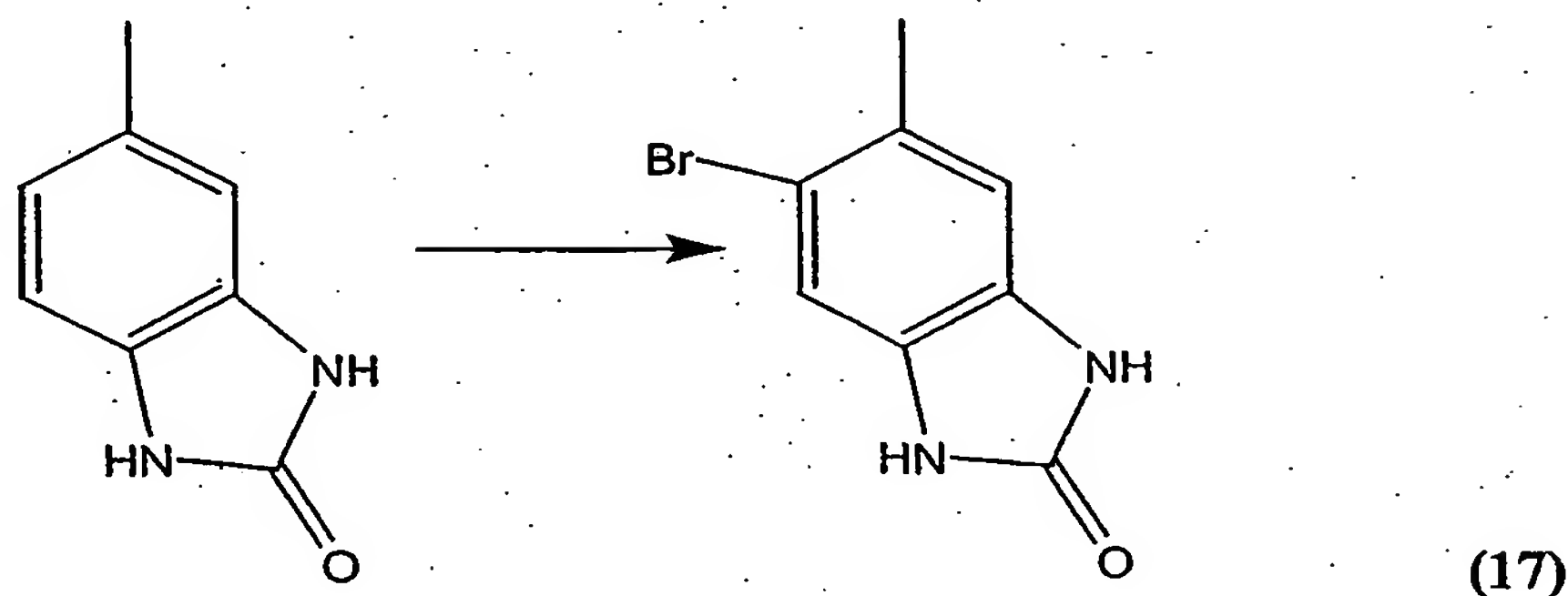
^1H NMR (CDCl_3): δ 8.70, 8.60 (2x bs, 2x 1H, 2x NH), 7.80 (d, 1H, ArH), 7.70 (s, 1H, ArH), 7.10 (s, 1H, ArH), 5.92 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz), 5.36 (m, 2H, H-3 + H-2), 5.23 (m, 1H, H-4), 4.33 (dd, 1H, H-6, J_{gem} 11.7 Hz, J_{vic} 4.6 Hz), 4.15 (d, 1H, H-6', J_{gem} 11.8 Hz), 3.96 (m, 1H, H-5), 2.07, 2.05, 2.0, 1.59 (4x s, 4x 3H, 4x OAc);

LRMS (ESI): m/z 526 $[\text{M}+\text{NH}_4^+]$;

$\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_{12}$: 508.43

Example 15:

5-Bromo-6-methylbenzimidazol-2-one (17)



A mixture of 6-methyl-1,3-dihydro-benzimidazol-2-one (1.00 g, 6.76 mmol) as prepared in Example 1, *N*-bromosuccinimide (1.30 g, 7.30 mmol) and 3-chloroperoxybenzoic acid (0.05 g, 0.29 mmol) in carbon tetrachloride (15 ml) was heated under reflux for 4 h. When cooled to room temperature, the mixture was filtered and the solid was recrystallised from methanol to give (17) as a beige solid (0.80 g). Furthermore,

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the solid insoluble in methanol was collected to give more (17) (0.50 g) and the solids were combined (1.30 g, 85%).

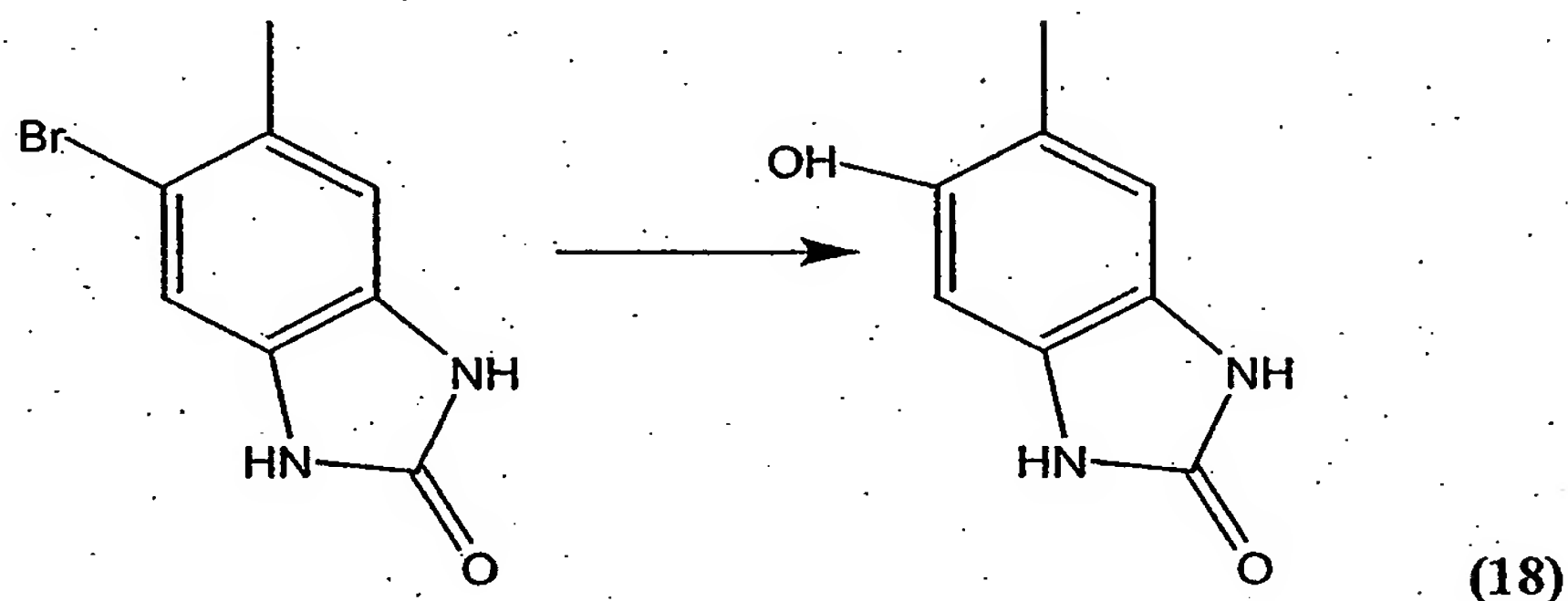
^1H NMR (d_6 -DMSO): δ 2.29 (s, 3H, CH_3), 6.89 (s, 1H, ArH), 7.06 (s, 1H, ArH), 10.59 (s, 1H, NH), 10.67 (s, 1H, NH).

5 ^{13}C NMR (d_6 -DMSO): δ 22.9 (CH_3), 111.0 (CH), 112.0 (CH), 114.8 (C), 128.9 (C), 129.7 (C), 130.0 (C), 155.7 (C).

ESMS: m/z 225.0 (M-1).

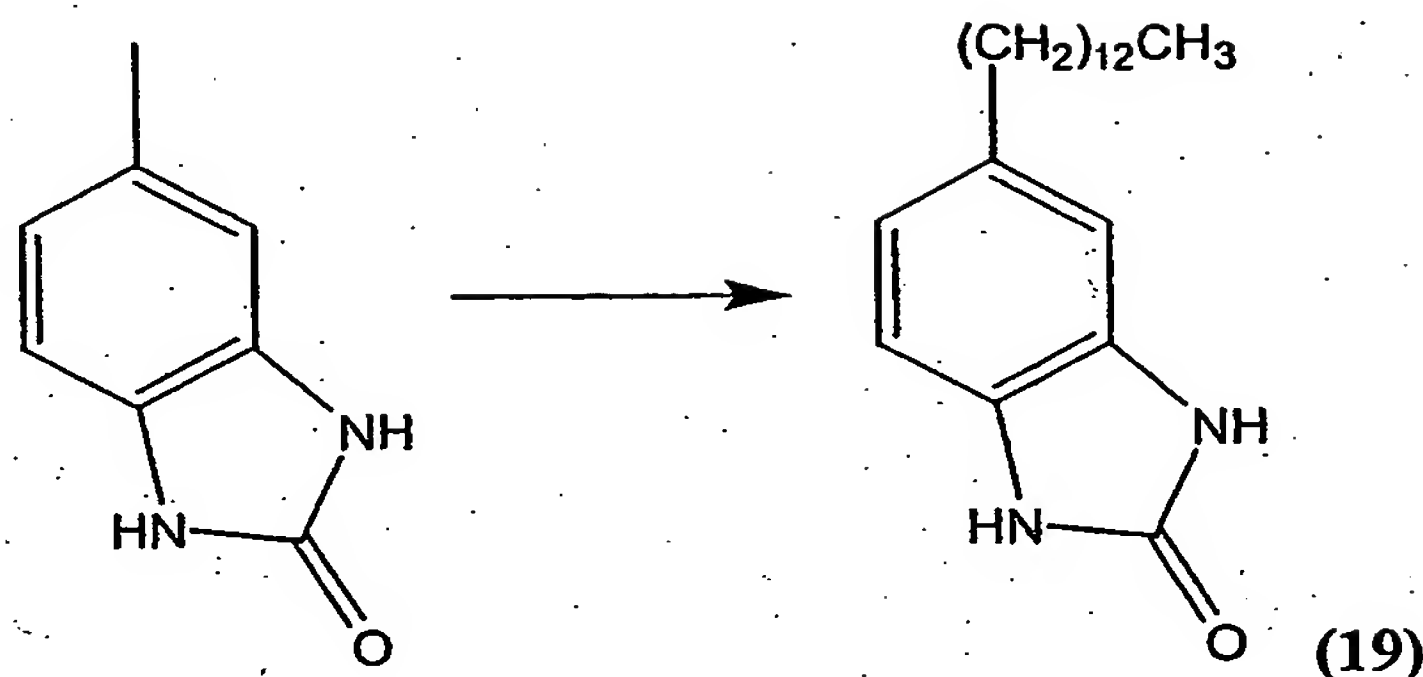
Example 16:

10 5-Hydroxy-6-methylbenzimidazol-2-one (18)



15 A solution of (16) (0.30 g, 1.32 mmol) in 10% sodium hydroxide (5 ml) and ethanol (5 ml) was heated under reflux for 1.5 h. When cooled to room temperature the ethanol was removed *in vacuo* and the mixture was filtered to give (18) as a white solid (0.20 g, 92 %).

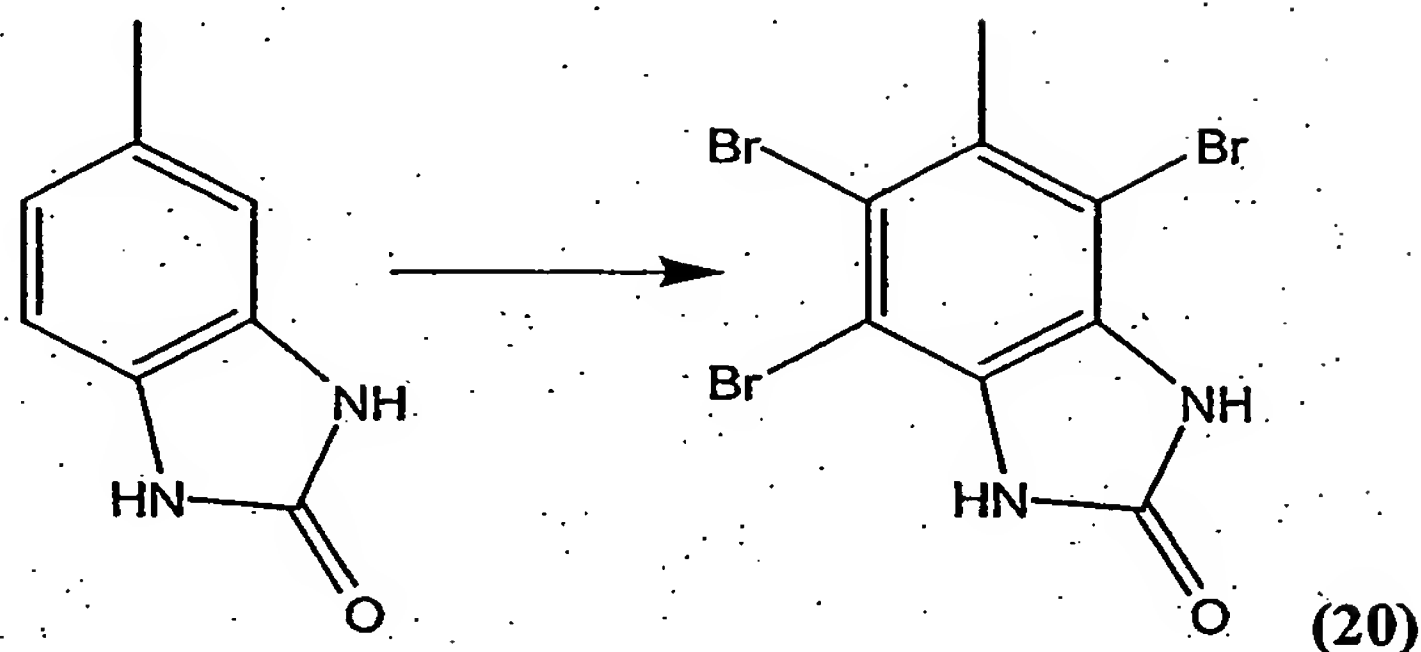
^1H NMR (d_6 -DMSO): δ 2.28 (s, 3H, CH_3), 6.88 (s, 1H, ArH), 7.05 (s, 1H, ArH), 10.61 (s, 1H, NH), 10.66 (s, 1H, NH).

Example 17:5-Dodecanylbenzoimidazol-2-one (19)

- 5 Sodium (0.05 g, 2.17 mg atom) was dissolved in ethanol (5 ml) and to this was added 6-methyl-1,3-dihydro-benzoimidazol-2-one (0.20 g, 1.35 mmol) and the solution was stirred at room temperature for 1 h. The solvent was removed *in vacuo* and the white solid was taken up in tetrahydrofuran (5 ml) and dimethyl sulfoxide (5 ml), and to this was added 1-bromododecane (0.34 g, 1.36 mmol) and the whole was stirred at room temperature for 2
- 10 h. *N,N*-Dimethylformamide (5 ml) was added to the mixture and the whole was stirred for a further 2 h. The mixture was filtered and the filtrate was evaporated *in vacuo* to give a yellow residue. The residue was placed on a silica column and chromatographed with 12.5% hexane- ethyl acetate to give (19) as a yellow oil.

¹H NMR (d_6 -DMSO): δ 0.84 (t, 3H, $J=4.5$ Hz, CH_3), 1.20 (s, 18H, 9 CH_2), 1.32-1.37 (m, 2H, CH_2), 1.74-1.79 (m, 2H, CH_2), 3.49 (t, 2H, $J=6.6$ Hz, CH_2), 6.71-6.76 (m, 3H, 3ArH), 10.45 (bs, 2H, 2NH).

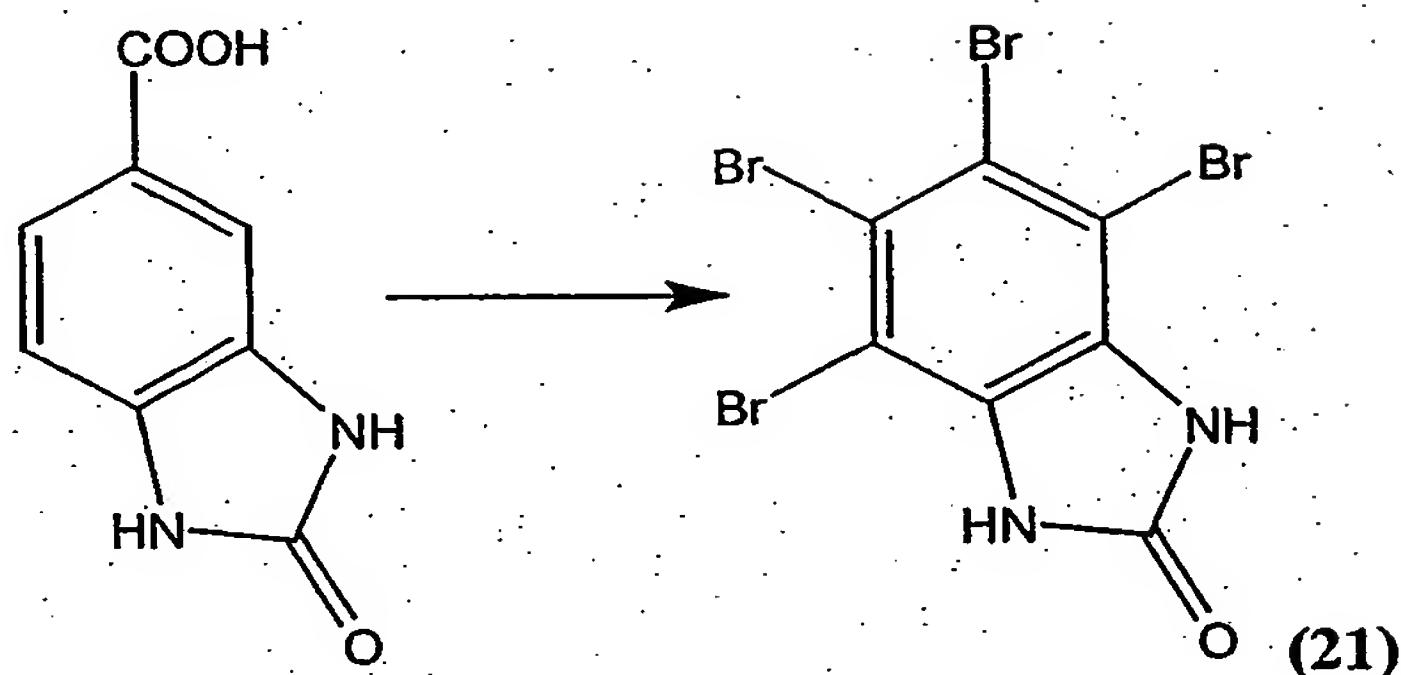
ESMS: m/z 317.3 ($\text{M}+1$).

Example 18:4,5,7-Tribromo-6-methylbenzimidazol-2-one (20)

A mixture of 6-methyl-1,3-dihydro-benzimidazol-2-one (0.50 g, 3.38 mmol), sodium acetate (1.70 g, 20.48 mmol) in acetic acid (4 ml) was heated at 70°C. A solution of bromine (1.60 g, 10.0 mmol) in acetic acid (5 ml) was slowly added over 10 min. The mixture was then heated under reflux for 1 h. When cooled to room temperature, the yellow mixture was poured into ice-cold water (100 ml) and filtered. The solid was recrystallised from ethanol to give (20) as a beige solid (0.76 g, 58%).

¹H NMR (d₆-DMSO): δ 2.56 (s, 3H, CH₃), 11.33 (s, 2H, 2NH).

¹³C NMR (d₆-DMSO): 30.8 (CH₃), 102.7 (C), 103.4 (C), 117.0 (C), 129.2 (CBr), 129.3 (CBr), 129.6 (CBr), 154.6 (C).

Example 19:4,5,6,7-Tetrabromobenzimidazol-2-one (21)

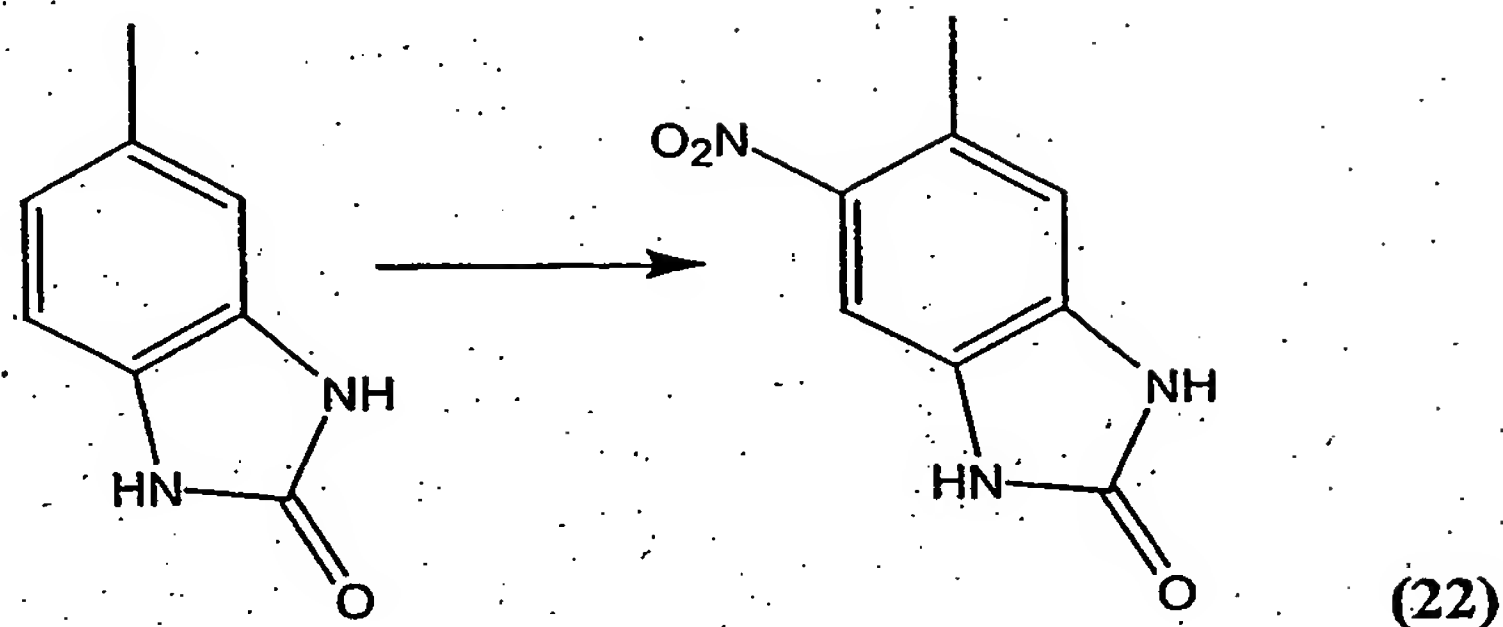
5

A mixture of 2-oxo-2,3-dihydro-1H-benzoimidazole-5-carboxylic acid (0.3 g, 1.7 mmol), sodium acetate (0.83 g, 10.0 mmol) in acetic acid (3 ml) was heated at 70°C. A solution of bromine (0.80 g, 5.0 mmol) in acetic acid (3 ml) was slowly added over 10 min. The mixture was then heated under reflux for 1 h. When cooled to room temperature, the yellow mixture was poured into ice-cold water (100 ml) and filtered. The solid was recrystallised from ethanol to give (21) as a golden-like solid (0.25 g, 33%).

¹H NMR (d₆-DMSO): δ 11.59 (s, 1H, COOH).

ESMS: *m/z* 444.8 (25%) (M-1), 446.9 (70%), 448.9 (100%), 450.9 (70%), 452.9 (15%).

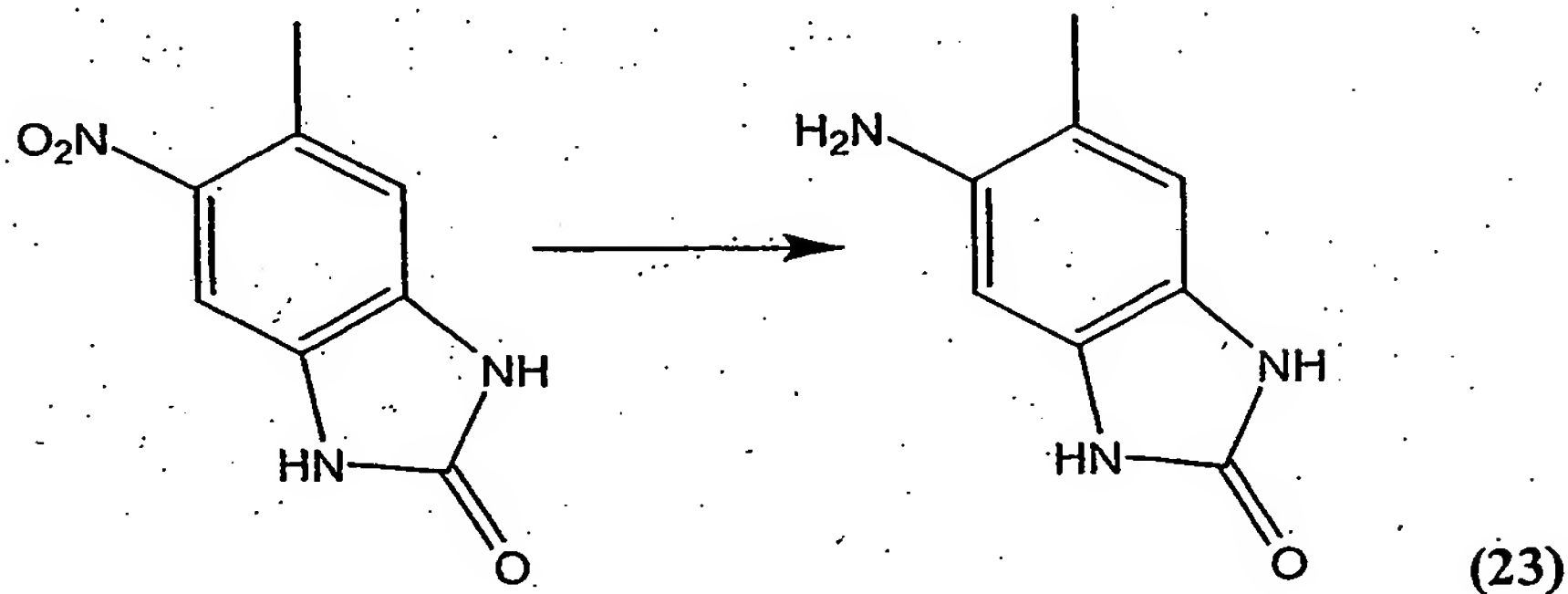
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Example 20:5-Methyl-6-nitrobenzimidazol-2-one (22)

- 5 A mixture of 6-methyl-1,3-dihydro-benzimidazol-2-one (0.30 g, 2.03 mmol) in 69% nitric acid (5 ml) was stirred over ice for 2 h. The yellow mixture was filtered and washed with water to give (22) as a bright yellow solid (0.39 g, 99.7%).

¹H NMR (d₆-DMSO): δ 2.53 (s, 3H, CH₃), 6.94 (s, 1H, ArH), 7.57 (s, 1H, ArH), 10.96 (s, 1H, NH), 11.20 (s, 1H, NH).

10

Example 21:5-Amino-6-methylbenzimidazol-2-one (23)

15

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A mixture of (22) (0.25 g, 1.30 mmol) and 10% palladium on carbon (0.05 g) in ethanol (15 ml) was hydrogenated at atmospheric pressure for 4 h. The catalyst was filtered off through Celite, and the solvent was removed *in vacuo* to give (23) as a white solid.

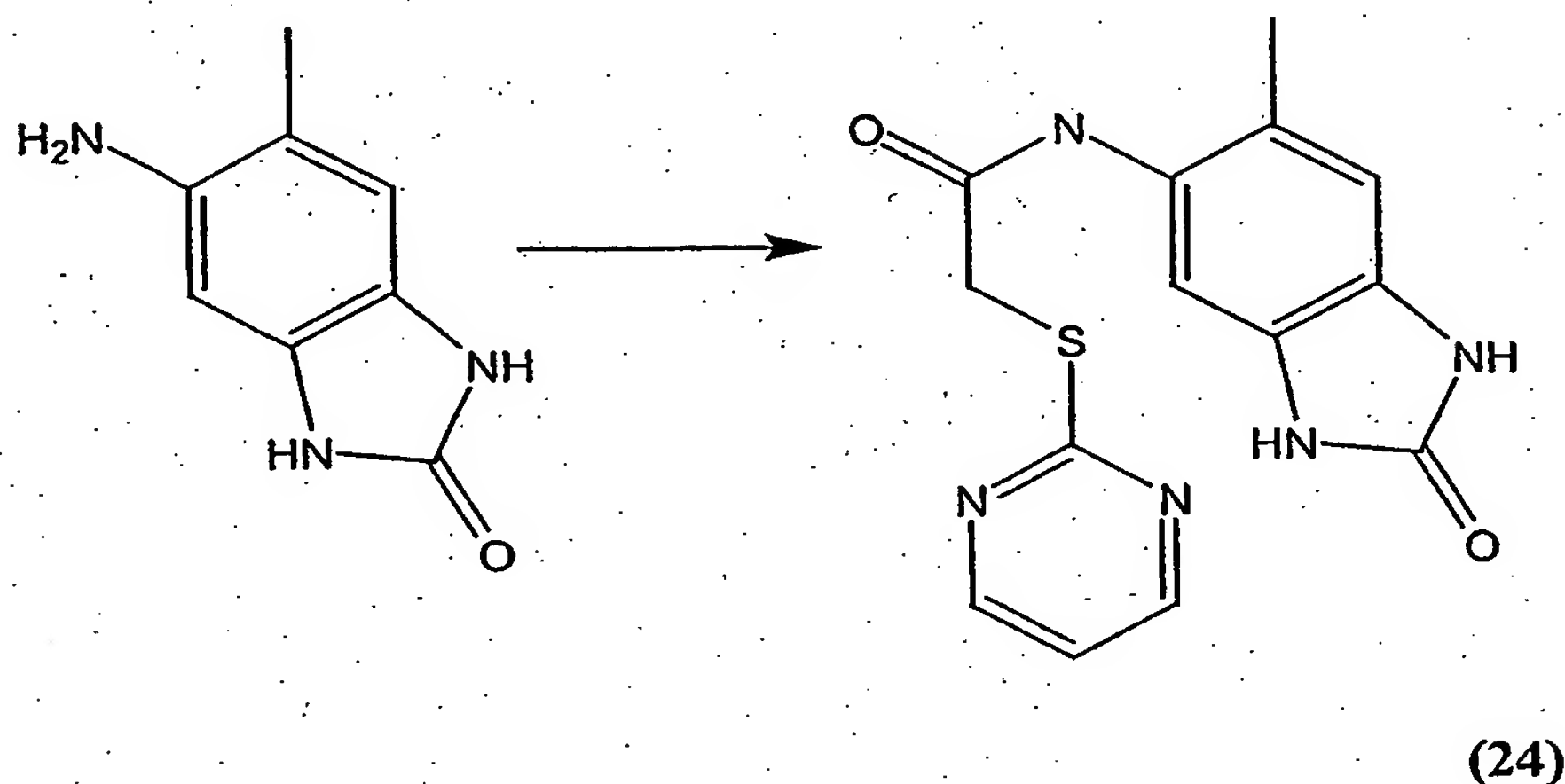
¹H NMR (d₆-DMSO): δ 2.01 (s, 3H, CH₃), 4.40 (s, 2H, NH₂), 6.29 (s, 1H, ArH), 6.50 (s, 1H, ArH), 9.96 (s, 1H, NH), 10.08 (s, 1H, NH).

ESMS: m/z 164.0 (M+1).

Example 22:

N-(6-Methylbenzimidazol-5-yl)-2-pyrimidin-2-yl-sulfanyl)-acetamide (24)

10



A solution of (2-pyrimidylthio)acetic acid (0.14 g, 0.82 mmol) and 1,1'-carbonyldiimidazole (0.24 g, 1.48 mmol) in dioxan (5 ml) was heated under reflux for 1 h. The solution was cooled, (23) (0.10 g, 0.61 mmol) was added, and the mixture was heated under reflux for 2 h. When cooled to room temperature, the mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was taken up in 10% potassium hydroxide (20 ml) and the mixture was filtered to give a dark brown solid. This was recrystallised from acetonitrile to give (24) as a brown solid.

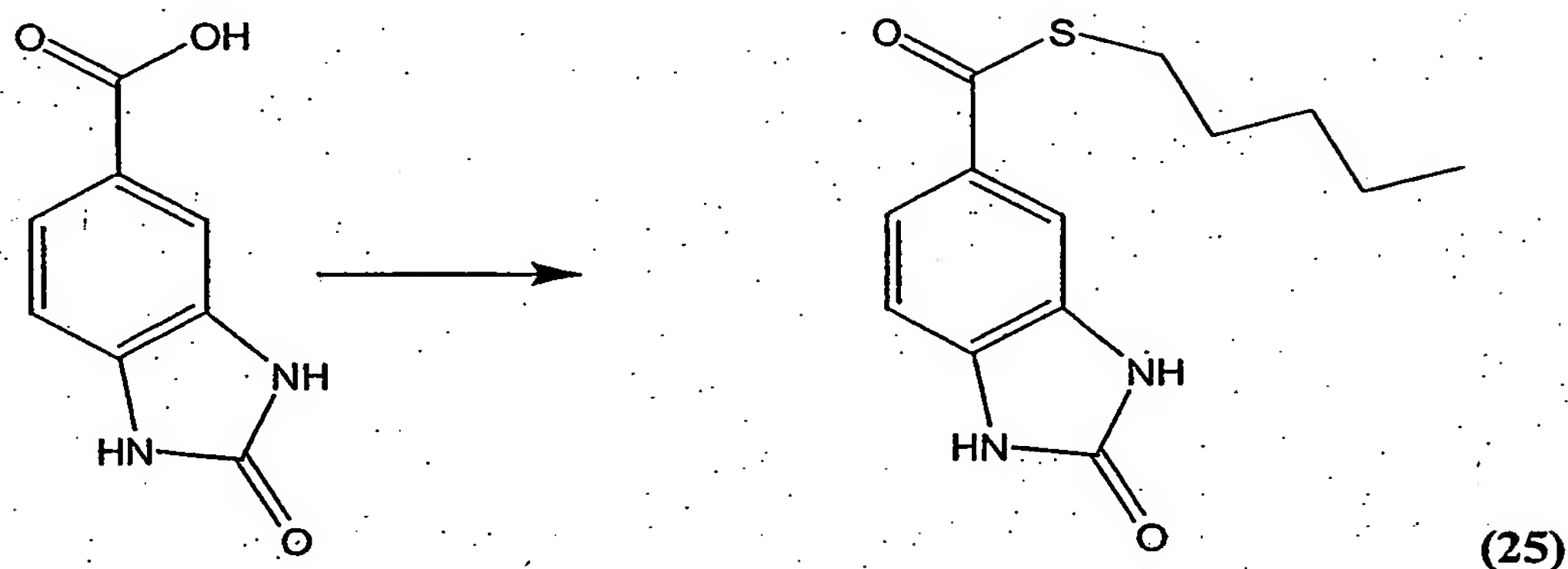
¹H NMR (d₆-DMSO): δ 2.20 (s, 3H, CH₃), 3.29 (s, 2H, CH₂), 6.71 (s, 1H, ArH), 7.30 (s, 1H, ArH), 7.93 (s, 1H, ArH), 8.56-8.65 (m, 2H, 2ArH), 10.33 (s, 1H, NH), 10.35 (s, 1H, NH).

ESMS: m/z 314.2 (M-1).

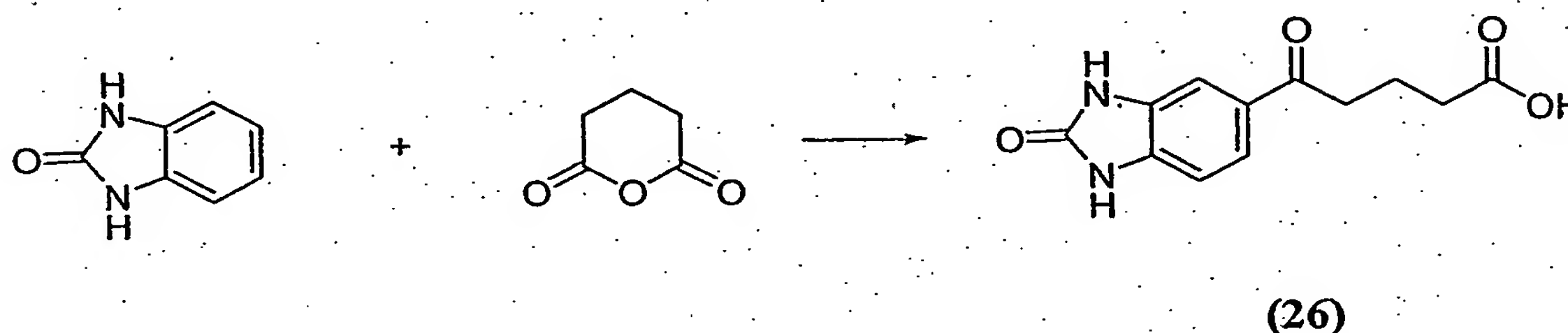
Example 23:

Pentyl-benzimidazol-2-one-5-carbothioate (25)

5



- 2-Oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylic acid (0.20 g, 1.12 mmol) in thionyl chloride (5 ml) was heated under reflux for 30 min. The excess of thionyl chloride was removed in vacuo and the residue was taken up in pyridine (5 ml). 1-Pentathiol (0.23 g, 2.21 mmol) was added and the whole was placed at reflux for 2 h. When cooled to room temperature, the mixture was filtered, and the filtrate was evaporated *in vacuo*. The residue was taken up in 10% potassium hydroxide and extracted with ethyl acetate, dried over sodium sulfate, and the solvent was removed *in vacuo* to give (25) as a white solid.
- 15 ¹H NMR (d₆-DMSO): δ 0.86 (t, 3H, $J=6.9$ Hz, CH₃), 1.31 (m, 4H, 2CH₂), 1.60 (m, 2H, CH₂), 2.99 (t, 2H, $J=7.2$ Hz, CH₂), 7.00 (d, 1H, $J=8.1$ Hz, ArH), 7.40 (s, 1H, H-4), 7.61 (s, 1H, $J=8.4$ Hz, ArH).

Example 24:5-(benzimidazol-2(3H)-one-6-yl)-5-oxopentanoic acid (26)

The procedure of Kosyakovskaya (17) was followed.

To a suspension of glutaric anhydride (3.42 g, 30 mmol) and benzimidazol-2(3H)-one (4.02 g, 30 mmol) in tetrachloroethane (100 ml), was added aluminium chloride from a freshly opened container (14.0 g, 105 mmol) in small portions. This mixture was heated to 120 C for 105 min, cooled to room temperature, then poured onto a mixture of ice (75 g) and concentrated hydrochloric acid (20 ml). The suspension was stirred overnight to break up solids that had formed. The tetrachloroethane was azeotropically distilled from the mixture with water under reduced pressure, leaving a brown solid suspended in an aqueous phase. The solid was filtered off and washed with dilute aqueous hydrochloric acid, then water. The solid was added to boiling aqueous sodium carbonate (5% w/v, 150 ml) and the solution filtered. Decolourizing charcoal was added to the cooled filtrate, which was then boiled for 5 min. The charcoal was removed by filtration, and the filtrate treated with concentrated hydrochloric acid until the pH of the solution was 4. The precipitated solid was allowed to stir overnight, filtered off, and dried in a vacuum oven at 50 C / 20 mmHg until a constant weight was achieved (1.6 g of brown solid). Nmr indicates that some starting material still remains. Recrystallization of a portion of this solid from acetic acid, as indicated by Kosyakovskaya, did not afford recovery of any product. A portion of the brown solid (300 mg) was dissolved in aqueous sodium bicarbonate (5% w/v, 10 ml) and insoluble material removed by filtration. The filtrate was acidified with concentrated hydrochloric acid and the precipitate formed filtered off and dried at the pump (30 mg).

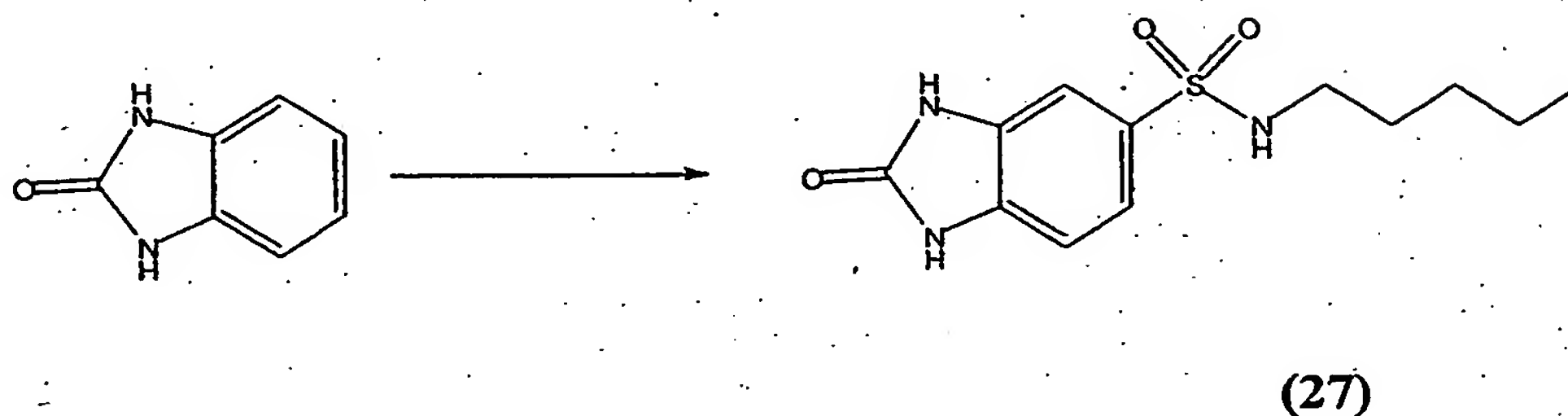
m/z (EI) found 248.0799, expected 248.0797 ($C_{12}H_{12}N_2O_4$) 248.0797;

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¹H-nmr (200 MHz, d₆-DMSO) δ 11.05 (brs, 1H), 10.89 (brs, 1H), 7.66 (dd, 1H), 7.47 (d, 1H), 7.00 (d, 1H), 3.00 (t, 2H), 2.29 (t, 2H), 1.81 (p, 2H).

Example 25:

5 2(3H)-benzimidazolone -5-sulfonic acid pentyl amide (27)

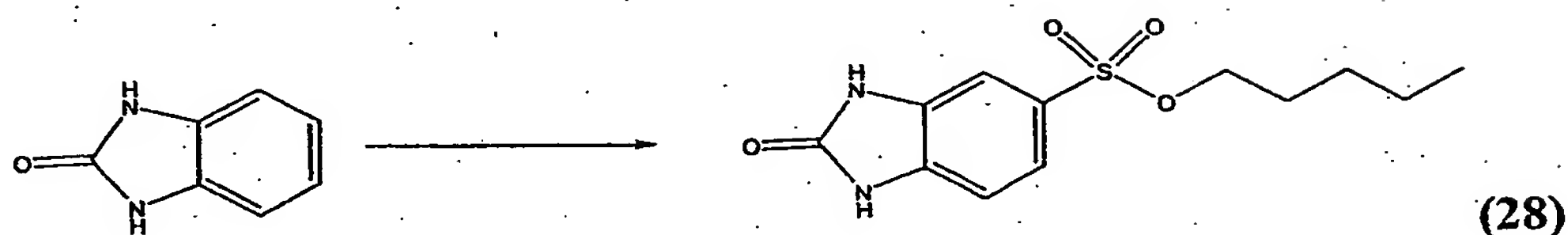


Chlorosulfonic acid (5 ml) was cooled to -5 C and 2(3H)-benzimidazolone (1 g) was added portionwise with stirring. The cooling bath was removed and the reaction allowed to warm to room temperature over 30 min, followed by heating to 95 C for an additional 30 min. After cooling to room temperature 2ml of the reaction solution was added to n-pentylamine (10 ml) in ice (20 g). Precipitation occurred and the suspension was allowed to stand over night. Filtration followed by extensive rinsing with water gave 760 mg of silvery-grey platelets. A small amount was recrystallised from methanol to give nearly colourless crystals of the title compound.

Mp 316-318 C;

m/z (EI) found 283.0992, expected (C₁₂H₁₇N₃O₃S) 283.0991; ¹H-nmr (500 MHz, d₆-DMSO) δ 11.01 (bs, 2H), 7.38 (dd, 1H), 7.34 (bt, 1H), 7.28 (d, 1H), 7.04 (d, 1H), 2.64 (m, 2H), 1.35-1.29 (m, 2H), 1.17-1.14 (m, 4H), 0.78 (t, 3H);

¹³C-nmr (125.8 MHz, d₆-DMSO) δ 155.35, 132.83, 132.35, 129.63, 119.91, 108.13, 106.59, 42.47, 28.53, 28.21, 21.61, 13.76.

Example 26:2(3H)-benzimidazolone-5-sulfonic acid pentyl ester (28)

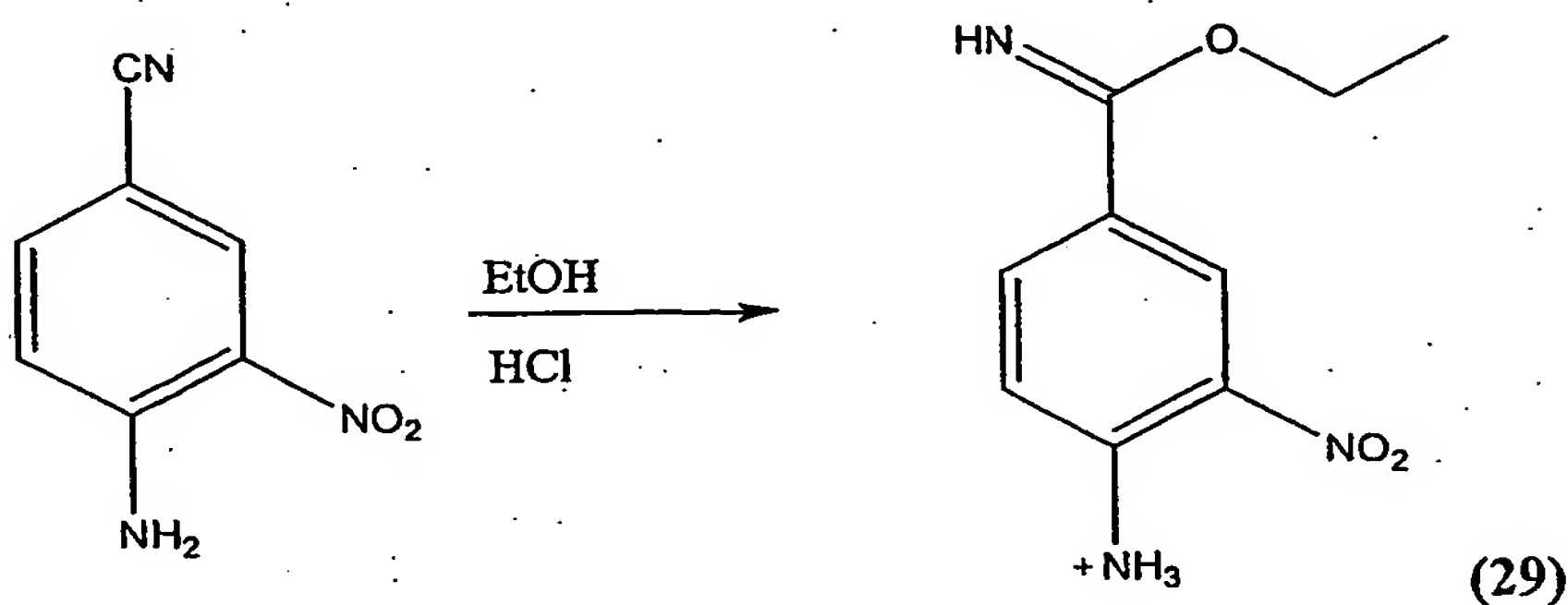
- 5 Similarly, a sample of the reaction mixture from Example 25 was quenched with n-pentanol instead of n-pentylamine. Chromatography using methanol resulted in partial decomposition of the sample, and recovered the title compound as a colourless solid (2.1 mg);

m/z (EI) found 284.0829, expected ($C_{12}H_{16}N_2O_4S$) 284.0831;

- 10 1H -nmr (200 MHz, d_6 -DMSO) δ 11.03 (bs, 2H), 7.38 (dd, 1H), 7.29 (d, 1H), 7.06 (d, 1H), 2.65 (m, 2H), 1.40-1.22 (m, 2H), 1.22-1.06 (m, 4H), 0.78 (t, 3H);

^{13}C -nmr (50.3 MHz, d_6 -DMSO) 155.27, 132.75, 132.27, 129.56, 119.856, 108.07, 106.53, 70.88, 70.82, 42.41, 28.46, 28.14, 21.54, 13.70.

- 15 Example 27:

Ethyl 4-amino-3-nitrobenzenecarboximidoate (29)

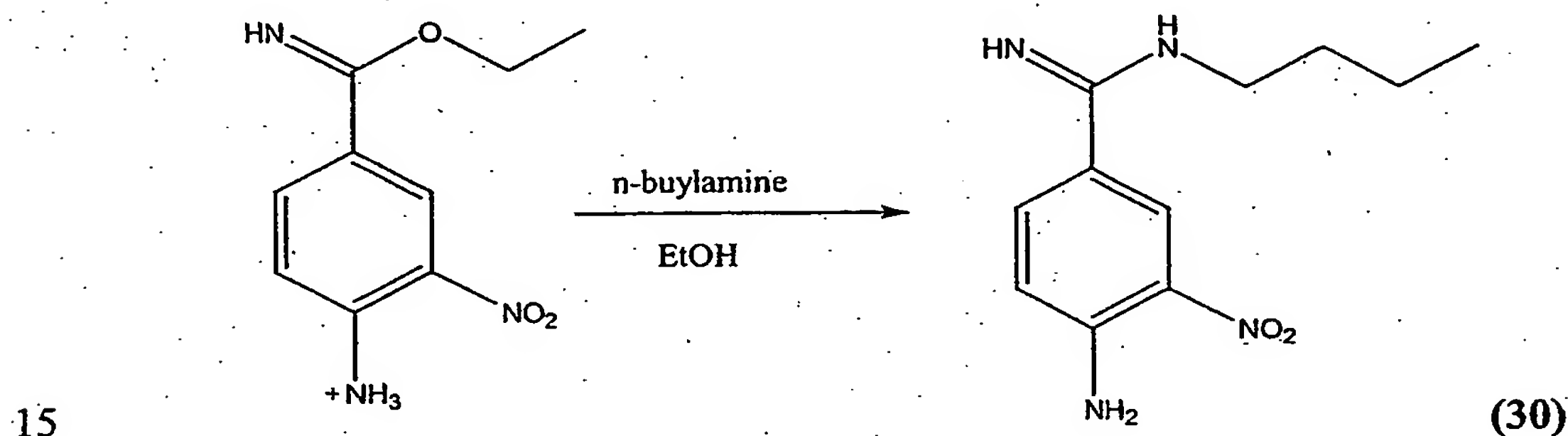
Synthesis of the nitrile to amidine was prepared according to the Pinner reaction (18).

4-Aminobenzonitrile (0.5 g, 3.1 mmol) was dissolved in 30 ml of dry ethanol and cooled in an ice/water bath. The solution was saturated with dry HCl and kept at a maximum temperature of 20°C. After 1hr of saturation the solution was allowed to stir at room temperature overnight. The resulting precipitate was collected and filtered to give the title product 0.55 g (86%) as a yellow powder which was pure enough to be used in the following reaction without further purification.

¹H NMR (DMSO) δ 1.46 (t, J 6.9 Hz, 3H, CH₃), 4.59 (q, J 6.9 Hz, 2H, O-CH₂), 7.17 (d, J 9.3 Hz, 1H, Ar-H), 8.09 (dd, J 2.1, 9.0 Hz, 1H, Ar-H) 8.81 (d, J 2.1 Hz, 1H, Ar-H).

Example 28:

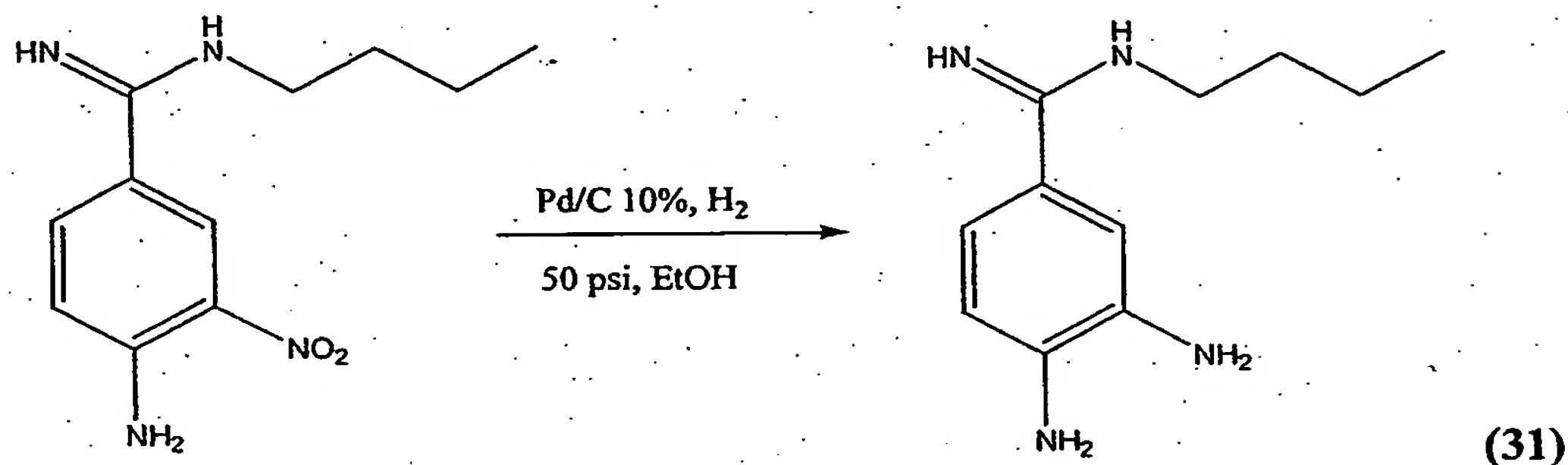
3-amino-N-butyl-3-nitrobenzenecarboximidamide (30)



n-Butylamine (0.292 g, 4.0 mmol) was distilled prior to use and was added to a stirred suspension of Ethyl 4-amino-3-nitrobenzenecarboximidate (0.7 g, 3.3 mmol) in 15 ml of dry ethanol. The mixture was stirred for 12 hrs at room temperature and then a further hour at 50°C. The resulting dark yellow precipitate was collected by filtration and recrystallised from boiling ethanol yield (89%) as yellow prisms.

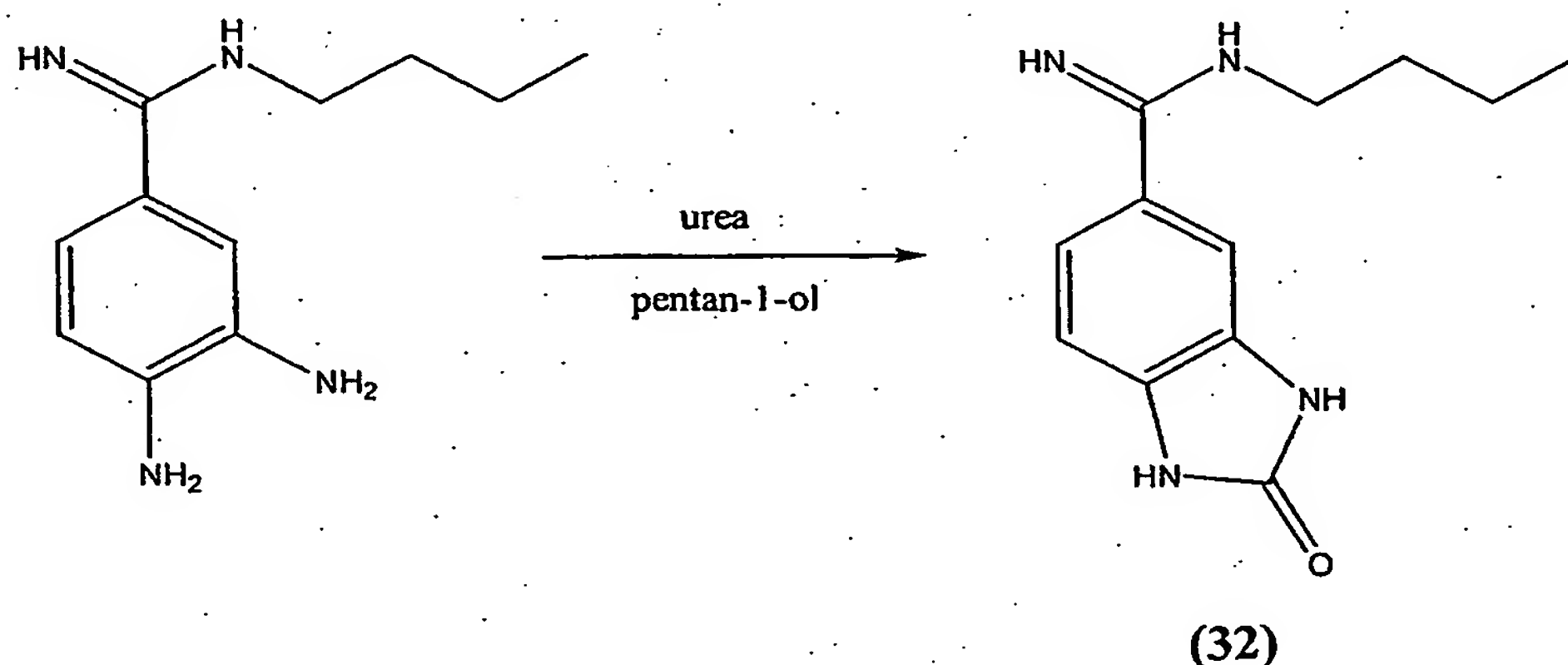
¹H NMR (DMSO) δ 0.92 (t, J 7.2 Hz, 3H, CH₃), 1.38 (m, 2H, CH₂), 1.59 (m, 2H, CH₂), 3.38 (m, 2H, NH-CH₂), 7.16 (d, J 9.0 Hz, 1H, Ar-H), 7.76 (dd, J 1.8, 8.7 Hz, 1H, Ar-H) 8.49 (d, J, 2.1, 1H, Ar-H). *m/z* (+ESI, 30V) 237.3 (MH⁺). (19)

25

Example 29:3,4-diamino-N-butylbenzenecarboximinamine (31)

3-amino-N-butyl-3-nitrobenzenecarboximidamide and 0.125 g of 10% Pd/C in 100 ml of dry methanol was subjected to hydrogenation at 50 psi for approximately 1h. The catalyst was filtered over Celite and washed with hot methanol. The filtrate was evaporated to give a sticky residue that was triturated with dry ether and dried under vacuum. The product was purified by recrystallising in boiling ethanol to give the title product as a purple powder 0.56 g (97%) mp 150.4°C.

¹H NMR (DMSO) δ 0.92 (t, J 7.2 Hz, 3H, CH₃), 1.38 (m, 2H, CH₂), 1.59 (m, 2H, CH₂), 3.35 (m, 2H, NH-CH₂), 6.91 (d, J 8.4 Hz, 1H, Ar-H), 7.32 (d, J 8.4 Hz, 1H, Ar-H) 7.43 (s, 1H, Ar-H). *m/z* (+ESI, 30V) 207.1 (MH⁺). (19)

Example 30:15 N-butyl-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboximidamide (32)

A solution of urea (0.15 g, 2.5 mmol) and 3,4-diamino-N-butylbenzenecarboximinamine (0.4 g, 1.9 mmol) in 10 ml of pentan-1-ol was vigorously stirred and heated to reflux under

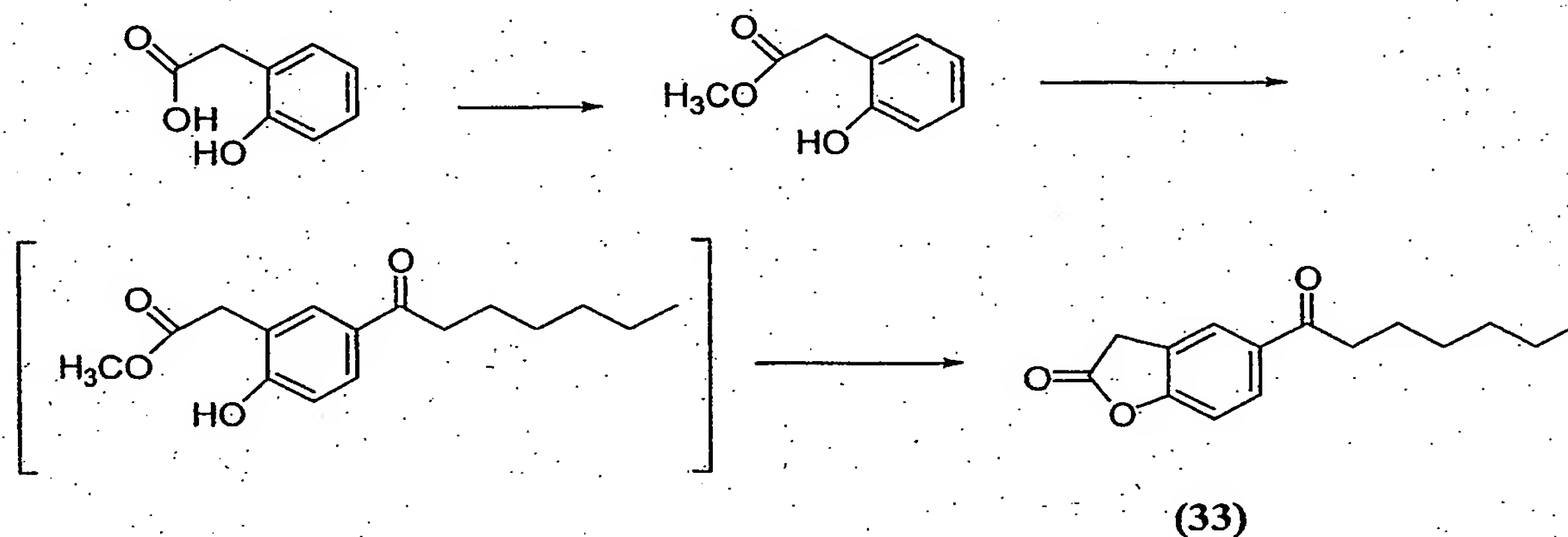
- 90 -

a nitrogen atmosphere. The heating was discontinued after 2 hours and on cooling to room temperature the solution was filtered. The filtrate was removed under reduced pressure to yield gummy oil which was triturated with dry ether and dried under vacuum to give the title product as a pink powder 0.3 g (66%) mp 168°C. (12)

- 5 ^1H NMR (DMSO) δ 0.92 (t, J 7.2 Hz, 3H, CH_3), 1.38 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 3.35 (m, 2H, NH-CH_2), 7.11 (d, J 8.1 Hz, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.37 (d, J 8.4 Hz, 1H, Ar-H). m/z (+ESI, 30V) 233.1 (MH^+).

Example 31:

- 10 5-heptanoylbenzofuran-2(3H)-one (33)



- 15 2-Hydroxyphenylacetic acid was converted to its methyl ester following the procedure in US4695648 (Pharmacia), then to the title compound by a modification of the Friedel-Craft procedure given in the same patent as follows.

To a solution of methyl 2-hydroxyphenylacetic acid methyl ester (1000 mg, 6.02 mmol) in nitrobenzene (10 ml) was added heptanoyl chloride (900 mg, 6.02 mmol), followed by
 20 aluminium chloride (1.6 g, 12.0 mmol, 2 equiv). The solution was heated to 60 C for 18 h, then cooled on ice before quenching with aqueous hydrochloric acid (1 M, 50 ml). The mixture was extracted with dichloromethane (3 x 50 ml) and concentrated. Chromatography on silica gel using ethyl acetate/petroleum spirits (20:80) as eluant gave a

semi-solid yellow residue. Recrystallization from dichloromethane/petroleum spirits at -15°C gave the title compound as a pale yellow solid (129 mg). Mp $64-67^{\circ}\text{C}$;

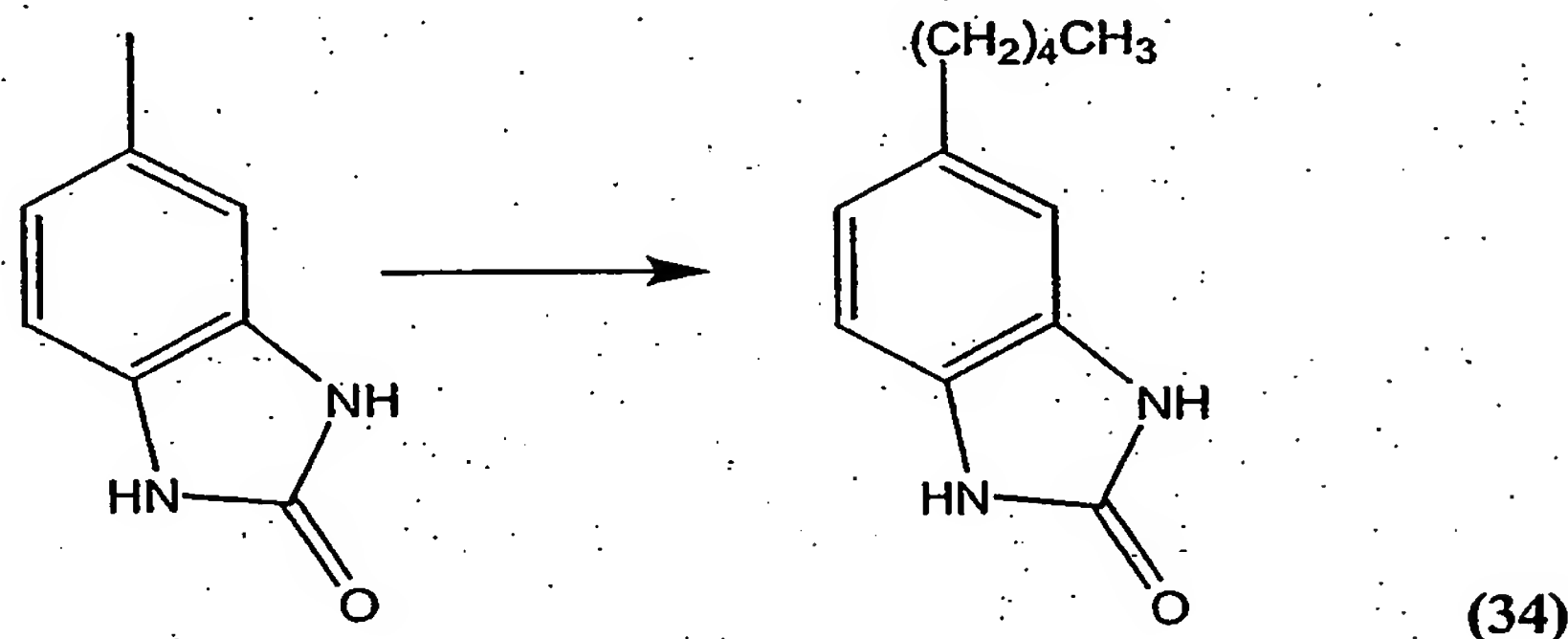
m/z (EI) found 246.1258, expected ($\text{C}_{15}\text{H}_{18}\text{O}_3$) 246.1256;

^1H -nmr (500 MHz, CDCl_3) δ 7.93 (d, 1H), 7.91 (s, 1H), 7.13 (d, 1H), 3.77 (s, 2H), 2.90 (t, 2H), 1.69 (p, 2H), 1.20–1.40 (m, 6H), 0.86 (t, 3H);

^{13}C -nmr (125.8 MHz, CDCl_3) δ 198.79, 173.10, 157.82, 133.48, 129.85, 124.69, 123.47, 110.46, 38.40, 32.53, 31.54, 28.91, 24.26, 22.42, 13.94.

Example 32:

5-Pentylbenzimidazol-2-one (34)



To a solution of 5-methylbenzimidazol-2-one (50 mg, 3.378×10^{-4} mol) in dry ethanol (5 ml), the freshly made sodium ethoxide in ethanol (1M, 1.01×10^{-3} mol equivalents) was added dropwise under a nitrogen atmosphere. At room temperature the mixture was stirred for 3 hrs under an atmosphere of nitrogen and the ethanol was then removed under reduced pressure. To this a solution of dry DMSO (3 ml) and 1-bromobutane (54 μl , 5.1×10^{-4} mol) was added and stirred at room temperature under an atmosphere of nitrogen overnight. The reaction mixture was then quenched with ice and neutralised using 1M hydrochloric acid solution, forming a white solid product. The solid was collected by filtration and purified using preparative thin liquid chromatography with 10% MeOH/ CHCl_3 .

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 $R_f = 0.78$ ESMS m/z 205 [(M + 1)/1]**Biological testing**

5

In vitro assay of MIF antagonism

The activity of compounds was studied in a bioassay utilising MIF-dependent activation of human dermal fibroblasts. Sampey *et al* (20) have shown that induction of the expression of cyclooxygenase-2 (COX-2) by the cytokine interleukin 1 (IL-1) is dependent upon the presence of MIF, i.e. can be prevented using specific anti-MIF monoclonal antibody. IL-1-induced COX-2 expression is therefore a MIF-dependent event.

S112 human dermal fibroblasts were propagated in RPMI/10% foetal calf serum (FCS). Prior to experimentation, cells were seeded at 10^5 cells/ml in RPMI/0.1% BSA for 18 hours. Cells were treated with recombinant human IL-1 (0.1 ng/ml) and with each compound at 1-100 μ M. A control was treated only with recombinant human IL-1 (0.1 ng/ml) and vehicle (DMSO). After 6 hours, cells were collected and intracellular COX-2 protein determined by permeabilisation flow cytometry. Cells permeabilised with 0.1% saponin were sequentially labelled with a mouse anti-human COX-2 monoclonal antibody and with sheep-anti-mouse F(ab)2 fragment labelled with fluorescein isothiocyanate. Cellular fluorescence was determined using a flow cytometer. At least 5000 events were counted for each reading, each of which was performed in duplicate, and the results expressed in mean fluorescence intensity (MFI) after subtraction of negative control-labelled cell fluorescence.

25

The effect of each compound was determined by subtracting the IL-1+compound-treated cell MFI from the IL-1-treated cell (control) MFI and expressed as % inhibition.

30

Results are shown in Table 1 below. In each case the % inhibition of IL-1-induced COX-2 expression is expressed as the mean, or mean \pm SEM where results are available from multiple experiments.

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Table 1.

Compound	% inhibition	Concentration (μ M)	number of expts.
5	52.2 +/- 2.1%	100	7
5	48.4 +/- 2.7%	50	23
5	34.0 +/- 5.3%	25	6
5	21.4 +/- 2.5%	10	8
5	7.7 +/- 2.0%	1	7
27	37.40%	50	1
15	33.10%	50	1
13	29.9 +/- 4.8%	100	3
13	32.2 +/- 4.7%	50	8
13	14.1 +/- 0.6%	10	3
13	9.6 +/- 3.0%	1	3
24	30.30%	50	1
22	30.00%	25	1
20	28.50%	25	1
19	24.50%	50	1
19	21.30%	25	1
17	22.40%	50	1
34	14.90%	10	1
8	19.9 +/- 3.9%	50	7
12	19.9 +/- 6.2%	50	4
10	19.3 +/- 4.1%	50	2
6	17.8 +/- 3.5%	50	4
11	17.5 +/- 5.1%	50	3
7	16.7 +/- 3.3%	50	2
9	14.9 +/- 5.8%	50	3
32	13.80%	50	1
2	13.0 +/- 3.3%	50	5
26	9.80%	50	1
23	8.50%	50	1
18	2.4 +/- 2.3%	50	2

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Figure 1 shows dose response curves for compound 5 observed in 3 experiments where compound 5 was added in 1 μ M, 10 μ M, 50 μ M and 100 μ M quantities and the samples analysed for IL-1 induced COX-2 expression as above.

5 *Effect of glucocorticoids on MIF antagonism*

In vitro assay of MIF antagonism in presence of glucocorticoid.

The above *in vitro* assay for analysing IL-1 induced COX-2 expression was repeated using Compound 5 (50 μ M) (column 1), dexamethasone (10^{-9} M) (column 2) or a combination of dexamethasone (10^{-9} M) and Compound 5 (50 μ M) (column 3). The results are shown in
10 Table 2 and Figure 2.

Table 2

Experiment	Compound	% Inhibition
1	Compound 5	45.1
2	Dexamethasone	46.8
3	Compound 5 + dexamethasone	73.6

15 *In vitro* assay for the effect of a MIF antagonist and a glucocorticoid on IL-1 induced phosphorylation of ERK.

S112 human dermal fibroblasts cultured in RPMI (serum-free) medium were stimulated with recombinant human IL-1 0.1 ng/ml, as described herein, for 30 minutes, with or without the addition of dexamethasone 10^{-9} M or benzimidazol-2-one-5-pentanoate
20 (compound 5) (50 μ M). The phosphorylation (activation) of ERK was assessed using Western blotting with a mAb specific for the phosphorylated (activated) form of ERK. In brief, cells were disrupted by repeated aspiration through a 21-gauge needle. After incubation on ice for 10 min and microcentrifugation at 3000 rpm for 15 min (4° C), the supernatants were removed, the protein concentration was determined, and the lysates were
25 stored at -80° C. Equal amounts of cellular proteins were fractionated on 10% SDS-polyacrylamide electrophoresis gels and transferred to polyvinylidene difluoride membranes. Immunoblotting was performed using antibodies directed against phospho-

p44/42 (ERK) and total p44/42 according to the manufacturer's instructions. The intensity of ERK activation is proportional to the size and optical density (darkness) of the resulting blots. Total ERK blots serve as a loading control, such that changes in phosphorylated ERK represent changes in phosphorylation and not in total ERK protein. In these experiments, ERK activation was demonstrated in response to IL-1 (lane 2) compared to untreated cells (control) (lane 1). ERK activation by IL-1 was not inhibited by benzimidazol-2-one-5-pentanoate (compound 5) (IL-1+MIF-a) (lane 3) or dexamethasone alone (not shown), but was powerfully inhibited by the combination of benzimidazol-2-one-5-pentanoate (compound 5) and dexamethasone (IL-1+MIF-a+DEX) (lane 4). The results are shown in Figure 3.

In vivo assay of MIF antagonism: Endotoxic shock

The activity of compound 5 was studied in the murine endotoxic shock model. This model has been previously shown to be dependent on MIF (21). Administration of a substance which inhibits the cytokine or biological activity of MIF would be expected to result in a reduction in serum levels of cytokines such as IL-1 or IL-6. Endotoxaemia was induced by intra-peritoneal injection of lipopolysaccharide (LPS) (15mg/kg) in 400 µl saline. Mice were treated with a saline solution (control) only, a saline solution and LPS, or LPS and compound 5 at a dose of 15 mg/kg body weight, administered by intra-peritoneal injection at 24 hours, 12 hours and 1 hour before intra-peritoneal LPS injection. After 1.5 hours mice were humanely killed by CO₂ inhalation then neck dislocation. Serum was obtained from blood obtained by cardiac puncture prior to death and measured for cytokines including interleukin 1 (IL-1) and interleukin 6 (IL-6) by ELISA. The production of IL-1 and IL-6 has been previously shown to be dependent on MIF (22). Macrophages were obtained by lavage of the peritoneal cavity using normal saline and placed into 24 well tissue culture plates for 18 hours in RPMI/10%FCS. The cultured peritoneal macrophage supernatants were then analysed for cytokines including IL-6. The peritoneal lavage supernatants were also analysed for cytokines including IL-6. The results are provided in Table 3 and Figure 4.

Table 3

Experiment	Serum IL-6 (ng/ml)	Cultured peritoneal macrophage IL-6 (ng/ml)	Peritoneal lavage IL-6 (ng/ml)
control	8.81	3.39	0
LPS only	261.05	3.40	16.11
LPS + compound 5	99.78	1.33	7.07

Figure 5 shows analysis of serum IL-1 (ng/ml) obtained from mice in which when LPS was administered alone or in combination with compound 5. A marked reduction in serum IL-1 concentration was observed in animals treated with compound 5.

The effect of compound 5 was tested under a variety of conditions in animals exposed to endotoxic shock induced as above by the injection of 15 mg/kg LPS by intraperitoneal injection. In each case compounds were administered by intraperitoneal injection at a dose of 15mg/kg. Compound 5 administration was associated with reductions in serum IL-1, IL-6, and TNF concentration whether administered by intraperitoneal injection (IP) or by oral gavage, and under a variety of administration regimens. These data suggest compounds of Formula I are active inhibitors of the biological or cytokine activity of MIF *in vivo*.

Table 4

Serum cytokine levels in response to LPS: effects of compound 5

Treatment regimen*	Result	Control	LPS	LPS + cpd	Inhibitory effect
IP injection 15 mg/kg -24, -6, -1h	Serum IL-1 at 1.5h	23.53	42.49	39.82	Y
Oral gavage 15 mg/kg -24, -6, -1h	Serum IL-1 at 1.5h		103.7	47.1	Y
IP injection 15 mg/kg -24, -6, -1h	Serum IL-1 at 6h		786.6	170.2	Y
IP injection	Serum IL-1 at 1.5h		484.8	283.8	Y

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15 mg/kg -24, -12, -1h					
IP injection 15 mg/kg -18h	Serum IL-1 at 1.5h		301.5	105.9	Y
Oral gavage 15 mg/kg -24h	Serum IL-1 at 1.5h	23.53	292.0	152.6	Y
Oral gavage 5 mg/kg -24h, -1h	Serum IL-1 at 1.5h		237.0	182.5	Y
Oral gavage 15 mg/kg -24h	Serum IL-6 at 1.5h	20360	121100	80250	Y
IP injection 15 mg/kg -24, -12, -1h	Serum IL-6 at 1.5h		92790	75260	Y
IP injection 15 mg/kg -24, -12, -1h	Serum IL-6 at 6h		261900	162800	Y
IP injection 15 mg/kg -1h	Serum IL-6 at 6h		215100	150300	Y
IP injection 5 mg/kg -1h	Serum IL-6 at 6h		215100	108200	Y
IP injection 1 mg/kg -1h	Serum IL-6 at 6h		215100	101000	Y
IP injection 15 mg/kg -18h	Serum TNF at 1.5h		4263	2422	Y

In vitro toxicity assay

The compounds of formula (I) may have low toxicity towards cells. The toxicity of compounds of formula (I) were examined *in vitro* to assess cytotoxicity. Human dermal fibroblast cell line (S112) cells were exposed to vehicle (control), compounds of formula (I) (50 μ M) or sodium nitroprusside (SNP) (0.5 μ M). SNP is a positive control agent which induces dose-dependent apoptosis in S112 cells. Toxicity was assessed by analysis of apoptosis using flow cytometric detection of cell surface Annexin V binding and propidium iodide staining. At least 5000 events were analysed for each experiment. Cells positive for both Annexin V and propidium iodide were designated as apoptotic and cells

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negative for both Annexin V and propidium iodide were designated as viable. Results are expressed as the percentage (%) of cells with each of these labels. No compound of formula (I) induced apoptosis at levels above the control whereas SNP induced a high level of apoptosis. The results for compound 5 are shown in Figure 6. The results for a number of compounds of formula (I) are shown in Figure 7.

In vitro assay of MIF antagonism: T cell activation

Activation of T lymphocyte responses is a critical event in the development of autoimmune and chronic inflammatory diseases. T lymphocyte activation in vitro and in vivo are known to be dependent upon the presence of bioactive MIF. For example, administration of specific monoclonal antibodies directed against MIF have been shown to inhibit development of T cell activation in vitro and of cutaneous delayed-type hypersensitivity responses in vivo (22) (7). The demonstration that compounds inhibitory of the cytokine and biological activity of MIF are inhibitory of T cell activation in vitro will be seen by those skilled in the art as supportive of the biological and functional antagonism of MIF provided by those compounds.

C57BL6/J male mice, aged 7-10 weeks old, were immunised with 200 µg of methylated bovine serum albumin (mBSA) dissolved in 20 µL of saline, emulsified in 200 µL of Freund's complete adjuvant (FCA) by subcutaneous injection. Seven (7) days later mice received a booster immunisation with 100 µg mBSA in 10 µL saline plus 100 µL FCA by subcutaneous injection. After a further seven (7) days mice were killed and spleens collected aseptically into Hanks buffered saline solution (HBSS). A single cell suspension was prepared in Petri dishes by flushing DMEM using a 26G needle and 2 mL syringe. The resulting cell suspension was centrifuged for 5-7 minutes and supernatant discarded. Erythrocytes were lysed using a solution containing 0.579% NH₄Cl, 0.000037% EDTA, and 0.1% NaHCO₃ in a 37 °C water bath. Tubes were then filled with DMEM and centrifuged for 5-7 minutes. The cell-containing pellet was then resuspended in DMEM containing 5% fetal calf serum (FCS) and 0.05% 2-mercapto-ethanol at a concentration of 1x10⁶ cells/mL and plated at 1x10⁵ cells/well in 96-well plastic tissue culture plates. Test substances (compound or vehicle) were added and incubated for 1 hour in a 37°C, 5%CO₂

incubator. The specific stimulating antigen, mBSA, was then added at 10-50 $\mu\text{g/mL}$ and plates incubated for 30 hours in a 37°C, 5%CO₂ incubator. Tritiated ³H-thymidine was then added at a concentration of 0.5 $\mu\text{Ci/well}$ for a further 18 hours. Cells were harvested on a Packard cell harvester, and the harvested material added to 750 $\mu\text{L/tube}$ scintillation fluid.

- 5 Scintillation counts were read on a Wallac beta-emission counter. Incorporation of ³H-thymidine into DNA is a measure of cell proliferation, and hence of antigen-specific T cell activation.

As shown in figure 8, T cell proliferation was significant increased in the presence of the specific sensitising antigen, mBSA, at 50 $\mu\text{g/mL}$. The addition of compound 5 in increasing concentrations exerted a dose-dependent and statistically significant inhibitory effect on antigen-specific T cell activation. In figure 8, asterisks signify a statistically significant result (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The concentration at which T cell activation was suppressed by 50% compared to vehicle-only-treated cells (EC₅₀) was calculated using Prism® software. The EC₅₀ for compound 5 in experiments where T cells were stimulated with 50 $\mu\text{g/ml}$ of mBSA was 13.75 μM .

Further compounds were also tested for their ability to inhibit antigen-specific T cell activation as a marker of the inhibition of the cytokine or biological activity of MIF using this assay. Table 5 lists the EC₅₀ for each compound in this assay, performed with concentrations of mBSA of either 50 or 10 $\mu\text{g/ml}$.

Table 5

Inhibition of T cell activation by compounds.

Compound	mBSA 50 $\mu\text{g/ml}$		mBSA 10 $\mu\text{g/ml}$	
	EC ₅₀ (μM)	no. expts	EC ₅₀ (μM)	no. expts
27	1.29	1	9.15	1
26	2.31	1	10.18	1
28	7.37	1	2.19	1
19	9.36	1	2.25	1
15	10.12	1	9.40	1
17	10.72	1	2.91	1
5	13.75	5	15.30	2
33	13.95	1	24.77	1

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16	25.99	1	23.84	1
18	33.98	1	19.31	1
7	44.76	3	33.89	1
34	49.89	1	13.50	1
11	50.73	3	44.44	1
9	61.92	3	76.96	1
13	71.73	4	53.28	1
10	77.54	3	>100	1
12	77.89	3	>100	1
8	>100	3	25.43	1
6	>100	3	25.84	1
19	>100	1	45.56	1
32	>100	1	>100	1
25	>100	1	>100	1
22	>100	1	>100	1
2	(not done)		7.29	2

In vivo assay of MIF antagonism: cutaneous delayed-type hypersensitivity (DTH) to mBSA

As noted above, cutaneous delayed-type hypersensitivity reactions (DTH) are an in vivo test of T cell activation. DTH reactions have been shown to be dependent on the presence of bioactive MIF, as shown by suppression of these reactions with monoclonal anti-MIF antibodies (22). A compound which inhibits the cytokine of biological activity of MIF may be expected to inhibit the development of DTH reactions in vivo. DTH reactions were induced in mice developing antigen-induced arthritis (see below). C57BL6/J male mice, aged 7-10 weeks old, were immunized on day 0 with 200 µg methylated BSA (mBSA) emulsified in 200 µl of Freund's complete adjuvant (FCA) injected subcutaneously into the flank skin. Mice were treated with compound 5, administered by intraperitoneal injection, once per 24 hours at a dose of 15 mg/kg body weight. After seven days, mice received 100µg mBSA and 100µl FCA by intradermal injection at the base of the tail. After a further 14 days, arthritis was induced by intra-articular injection of 30 µg mBSA in 10 µl of sterile saline into the left knee, the right knee being injected with sterile saline alone. DTH reactions were induced as follows: these mice were challenged 24 h before the end of the experiment by intradermal injection of 50 µg mBSA in 20 µl saline into one hind footpad. A similar volume of saline was injected into the contralateral footpad as a control. Footpad swelling was quantified 24 h later using a micrometer. DTH was recorded as the

difference in skin swelling between mBSA and saline-injected footpads, and expressed as change in footpad thickness (Δ mm). A reduction in skin thickness is consistent with an inhibitory effect on the biological or cytokine activity of MIF.

As shown in figure 9, a statistically significant reduction in cutaneous DTH reactions was observed in mice treated with compound 5, compared to mice treated with vehicle ($p < 0.05$).

In vivo assay of MIF antagonism: antigen-induced arthritis.

Rheumatoid arthritis is a common, serious, chronic inflammatory disease affecting synovial joints, of which the etiology is unknown. Rheumatoid arthritis is one of the most common autoimmune or chronic inflammatory diseases, and can be seen as a model for other, less common, autoimmune and chronic inflammatory diseases. MIF has been confirmed as an important mediator in several animal models of rheumatoid arthritis, through studies in which antagonism of MIF with a monoclonal anti-MIF antibody exerted significant inhibitory effects on disease (23), (24), (8). Included among the animal models of rheumatoid arthritis in which MIF has been shown to be an essential factor is murine antigen-induced arthritis (8). A compound which inhibits the cytokine or biological activity of MIF might be expected to inhibit the development of murine antigen-induced arthritis in vivo.

Antigen-induced arthritis was induced in mice. C57BL6/J male mice, aged 7-10 weeks old, were immunized on day 0 with 200 μ g methylated BSA (mBSA) emulsified in 200 μ l of Freund's complete adjuvant (FCA) injected subcutaneously into the flank skin. Mice were treated with compound 5, administered by intraperitoneal injection, once per 24 hours at a dose of 15 mg/kg body weight. After seven days, mice received 100 μ g mBSA and 100 μ l FCA by intradermal injection at the base of the tail. After a further 14 days, arthritis was induced by intra-articular injection of 30 μ g mBSA in 10 μ l of sterile saline into the left knee, the right knee being injected with sterile saline alone.

Arthritis was analysed histologically at day 28 after first immunisation. Knee joints were dissected and fixed in 10% buffered formalin for 7 days. Fixed tissues were decalcified for

3 weeks in 15% ethylene-diamine-tetra-acetic acid (EDTA), dehydrated and embedded in paraffin. Sagittal sections (5 μ m) of the knee joint were stained with Safranin-O and counterstained with fast green /iron hematoxylin. Histological sections were scored from 0 to 3 for four parameters: *Synovitis* was defined as hyper-cellularity of the synovium including pannus formation. *Joint space exudate* was identified as leukocytes, discretely or in aggregates, in the joint space. *Cartilage degradation* was defined as the loss of Safranin-O staining of articular cartilage (0 = full stained cartilage, 3 = totally unstained cartilage). *Bone damage* was defined as the extent and depth of the subchondral bone invasion by pannus. A total score was also generated from the sum of these four parameters (maximum 12).

The results of treating mice with compound 5 are shown in figure 10. In figure 10a, the total arthritis score for vehicle and compound-treated animals is presented graphically. A statistically significant reduction in total arthritis score is seen ($p = 0.0024$). In figure 10b, individual parameters of arthritis are presented graphically. Statistically significant reductions in the severity of all individual parameters of arthritis can be seen for animals treated with compound 5.

An additional compound of Formula I has also been tested in the murine antigen induced arthritis model. Results for compound 13 are provided in table 6. Compound 13 also exerted an inhibitory effect on the severity of murine antigen induced arthritis.

Table 6

Effects of compound 13 in murine antigen induced arthritis.

	Total arthritis score (mean \pm SEM)	Number of experiments
vehicle	6.25 \pm 0.2	2
compound	5.17 \pm 1.9	3

In vivo assay of MIF antagonism: ex vivo T cell activation

As MIF is important in T cell activation, a compound capable of inhibiting the cytokine or biological activity of MIF might be expected to exert inhibitory effects on T cell responsiveness. In vivo administration of such a compound might be expected to exert effects on T cell responsiveness even after the T cells have been removed from exposure to the compound, that is, if T cells were studied ex vivo after in vivo treatment with the MIF antagonist compound. To measure ex vivo antigen-specific T cell activation, draining lymph nodes from arthritic limbs or spleens were removed from mice with murine antigen induced arthritis, induced as above with mBSA, at day 28 after first immunisation and a single cell suspension prepared in DMEM containing 5% FCS and 0.05% 2-mercaptoethanol. 1×10^5 cells /200 μ l were cultured in triplicate in the presence or absence of mBSA (0.1, 1.0, 10 μ g /ml) in 96-well plates for 48 hours (37°C, 5% CO₂). The T cell proliferation response was determined by measuring ³H-thymidine incorporation during the final 18 hr. The cells were harvested and radioactivity incorporation into the DNA was measured with a Wallac 1409 liquid scintillation counter. The means of each triplicate culture were calculated. Each experiment comprised at least three individual animals and the results presented represent the mean \pm SEM of groups of animals in each experiment. The percentage inhibition of T cell proliferation was calculated using the result of the ³H-thymidine incorporation of cells from compound-treated animals subtracted from the ³H-thymidine incorporation of cells from vehicle-treated animals.

Figure 11a depicts graphically the effect of in vivo treatment with compound 5 on the ex vivo activation of lymph node T cells in response to mBSA. As can be seen, cells from mice treated in vivo with compound 5 (filled bars) exhibited reduced proliferative responses to mBSA compared to cells from mice treated with vehicle. Representative of at least three separate experiments, each in triplicate and each including several individual animals.

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Figure 11b depicts graphically the effect of in vivo treatment with compound 13 on the ex vivo activation of splenic node T cells in response to mBSA. As can be seen, cells from mice treated in vivo with compound 13 (hatched bars) exhibited reduced proliferative responses to mBSA compared to cells from mice treated with vehicle. As can be seen from table 7, in vivo administration of compound 13 and compound 5 each exert an inhibitory effect on ex vivo splenic T cell proliferation.

Table 7

Inhibition of ex vivo splenic T cell activation by in vivo treatment with compounds.

Compound	% inhibition	[mBSA] ($\mu\text{g/mL}$)	no. expts
13	28%	10	1
5	30%	10	3
5	33%	1	3

In vitro assay of MIF antagonism: dermal fibroblast proliferation induced by recombinant MIF.

It is well known to those skilled in the art that MIF is able to induce proliferation in a number of cell types including cells derived from patients with rheumatoid arthritis (25). It has also been demonstrated that antagonism of MIF with a monoclonal anti-MIF antibody can inhibit the proliferation of cells in vitro. A compound with the ability to inhibit the cytokine or biological function of MIF might be expected to inhibit the proliferative effect of MIF.

20

The activity of compound 5 was studied in a bioassay utilising MIF-induced proliferation of human dermal fibroblasts. S112 human dermal fibroblasts were propagated in RPMI/10% foetal calf serum (FCS). Prior to experimentation, cells were seeded at 10^5 cells/ml in RPMI/0.1% BSA for 18 hours. At time point zero, culture medium was replaced with RPMI/10% FCS and treatments administered. Cells were treated with recombinant human macrophage migration inhibitory factor (MIF) 50 ng/ml and/or compound 5 at a 1–1000 molar ratio to the concentration of MIF. At a time point 30 hours later, cells were pulsed with 1 μCi /well of ^3H -thymidine. At a time point 48 hours after

25

commencement of the experiment, cells were harvested using a semi-automated cell harvester. The radioactivity incorporated into DNA was determined by liquid scintillation counting, with results expressed as [^3H] thymidine incorporation.

- 5 Figure 12 depicts graphically the effect of compound 5 (0.013 μM) on proliferation of S112 cells treated with recombinant human MIF. A marked and statistically significant inhibitory effect was observed. The data presented are representative of four separate experiments.

10 In table 8, the inhibitory effect of compound 5 is expressed as the % inhibition of proliferation compared to the proliferation of rhMIF-treated cells.

Table 8

Inhibition of MIF-induced proliferation of s112 dermal fibroblast by compound 5.

Compound	% inhibition	concentration (μM)	p value	no. expts
5	98%	0.013	P<0.001	4

15 ***In vitro assay of MIF antagonism: inhibition of peritoneal macrophage cytokine production.***

MIF is known to be a participant in the innate immune response to toxins such as the bacterial endotoxin lipopolysaccharide (LPS). As shown above, antagonists of MIF can
20 inhibit endotoxin-induced macrophage cytokine production in vivo. A compound with the ability to inhibit the cytokine or biological function of MIF might be expected to inhibit the activation of cytokine production by macrophages in response to LPS.

C57BL6/J male mice were injected intraperitoneally with 2ml of thioglycollate. Five (5)
25 days later peritoneal macrophages were collected by lavaging the peritoneum of anaesthetized mice with 3ml of cold Hanks buffered saline solution. Cells from several mice were pooled, washed and re-suspended in DMEM supplemented with 5%FCS. Cells were plated in 96 well plastic tissue culture plates at 1×10^5 cells/well. Cells were treated with compound or vehicle for 1 hour in a 5% CO_2 incubator at 37 $^\circ\text{C}$. Cells were then

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treated with LPS (10-100 ng/ml) or recombinant human interferon- γ (10 units/ml) and incubated for 24 hours. After 24 hours, 50 μ l of supernatant from each well was carefully removed and transferred to ELISA plates. The concentration of interleukin 1 (IL-1) or interleukin 6 (IL-6) was measured by ELISA. The concentration of compound at which LPS- or interferon- γ -induced cytokine release was suppressed by 50% compared to vehicle-only-treated cells (EC50) was calculated using Prism® software. Table 9 lists the EC50 for two compounds tested in this assay.

Table 9

10 Inhibition of murine peritoneal macrophage cytokine production.

Compound	Interferon- γ (units/ml)	[LPS] ng/mL	EC50 (μ M)	no. expts
IL-1				
13		100	16.67	1
5		10	0.075	2
IL-6				
13	10		9.82	1
5		100	17.72	2

In vitro assay of MIF antagonism: inhibition of peritoneal macrophage nitric oxide release.

MIF is able to induce or facilitate the expression and release of a wide variety of pro-inflammatory and/or destructive molecules. In the case of macrophages, in addition to the facilitation of cytokine release, MIF is able to facilitate the release of nitric oxide (NO) (26). A compound with the ability to inhibit the cytokine or biological function of MIF might be expected to inhibit the activation of NO production by macrophages.

20 C57BL6/J male mice were injected intraperitoneally with 2ml of thioglycollate. Five (5) days later peritoneal macrophages were collected by lavaging the peritoneum of anaesthetized mice with 3ml of cold Hanks buffered saline solution. Cells from several mice were pooled, washed and re-suspended in DMEM supplemented with 5%FCS. Cells were plated in 96 well plastic tissue culture plates at 1×10^5 cells/well. Cells were treated
25 with compound or vehicle for 1 hour in a 5% CO₂ incubator at 37 °C. Cells were then

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treated with LPS (10 ng/ml) and recombinant human interferon- γ (10 units/ml) and incubated for 24 hours. After 24 hours, 50 μ l of supernatant from each well was carefully removed and transferred to ELISA plates. The concentration of nitrite in culture supernatants was measured by the Greiss assay (27). The results were defined as the percentage inhibition of nitrite concentration in compound-treated cell culture supernatants compared to that of vehicle-treated cells.

Table 10 displays the results for two compounds tested in this assay. Marked and statistically significant reductions in nitrite concentration were observed in the supernatants of cells treated with compounds.

Table 10

Inhibition of murine peritoneal macrophage nitric oxide production.

Compound	Concentration (μ M)	% concentration inhibition from control	Nitrite from	P value
11	25 μ M	3.1 +/- 1.2%		
	50 μ M	28.1 +/- 1.0%		P<0.001
	100 μ M	65.5 +/- 0.8%		P<0.001
5	25 μ M	11.1 +/- 1.3%		P<0.001
	50 μ M	19.7 +/- 1.6%		P<0.001
	100 μ M	28.6 +/- 1.5%		P<0.001

***In vitro* assay of MIF antagonism: inhibition of human rheumatoid arthritis synovial fibroblast proliferation.**

MIF is known to be a stimulus or participant in the proliferation of multiple cell types with relevance to disease states. For example, this been shown to be the case for the native resident cells of the synovial lesion of human rheumatoid arthritis, namely fibroblast-like synoviocytes (FLS). Lacey *et al* (26) reported that recombinant MIF induces proliferation of FLS, and moreover that a monoclonal anti-MIF antibody is able to suppress the proliferation of FLS induced by another cytokine, interleukin 1 (IL-1). A compound

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capable of inhibiting the cytokine or biological activity of MIF would be expected to be able to inhibit the IL-1-induced proliferation of human rheumatoid arthritis FLS.

Fibroblast-like synoviocytes (FLS) were obtained from synovium of rheumatoid (RA) patients undergoing joint replacement surgery and prepared according to Lacey *et al* (26). FLS were isolated using enzyme digestion and cultured in RPMI/10% fetal calf serum (FCS) as previously described. A single cell suspension was obtained by digesting minced synovial tissue with 2.4 mg/ml Dispase (grade II, 5 U/mg) 1 mg/ml collagenase (type II) and DNase (type I). FLS were propagated in 10 cm culture plates in RPMI supplemented with 10 % FCS at 37°C in a 5% CO₂ humidified incubator. Cells beyond 3rd passage were more than 99% CD45-. Cells were used between passages 4 and 9. Cells from four individual human RA donor FLS used.

To determine the effect of compound 5 on FLS proliferation, DNA synthesis was measured by [³H] thymidine incorporation. FLS were seeded overnight at 0.5x10⁵ cells per well in 24 well tissue culture plates in RPMI/10% FCS. Cell growth was synchronised by culturing FLS in RPMI/0.1 % bovine serum albumin for 24 h. FLS were treated with human recombinant IL-1β (0.1 ng/ml) and compound 5 (50 μM) or vehicle for 54 h prior to cells being pulsed for 18 h with 1 μCi/ml ³H-thymidine. Duplicate or triplicate cultures were used for each determination as noted. FLS were detached using Trypsin-EDTA, harvested using a cell harvester, and the radioactivity incorporated into DNA determined using a Wallac 1409 liquid scintillation counter. Results were expressed as the percentage of control (untreated) cell proliferation, and the mean ± SEM of four experiments calculated.

As shown in Table 11, IL-1 statistically significantly induced the proliferation of human rheumatoid arthritis FLS. There was no effect of vehicle on proliferation, but compound 5 treatment was associated with a marked and statistically significant inhibition of IL-1-induced FLS proliferation.

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These data are consistent with compound 5 being an inhibitor of the cytokine and biological activity of MIF.

Table 11.

5

Control	IL-1	IL-1 + vehicle	IL-1 + compound 5
100±0	167±39	156±32	18±6

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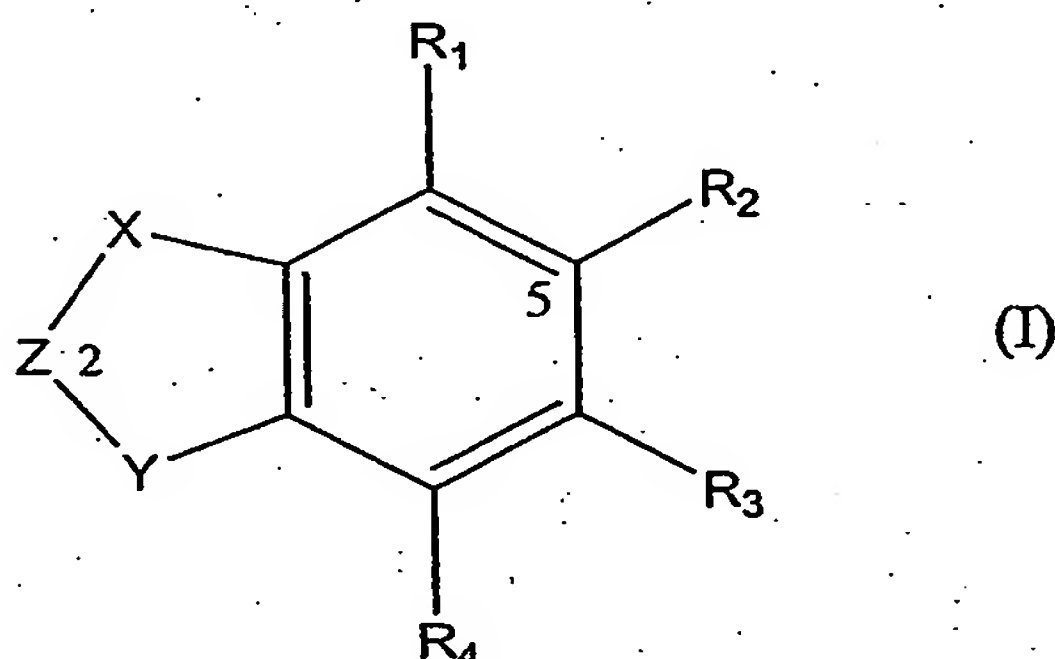
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CLAIMS:

1. A method of inhibiting cytokine or biological activity of MIF comprising contacting MIF with a cytokine or biological activity inhibiting effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof



wherein

10 X is selected from -O-, -S-, -C(R₅)(R₅)- or -N(R₆)-;

Y is selected from -N(R₇)-, -O-, -S- or -C(R₇)₂-;

Z is selected from -C(O)-, -C(S)-, -C(=NR₆)-, -S(O)- or -S(O)₂-;

15

R₁ is selected from hydrogen, C₁₋₃alkyl, (CR₅R₅)_nOR₇, (CR₅R₅)_nSR₇, (CR₅R₅)_nN(R₆)₂ and (CR₅R₅)_nhalo;

20 R₂ is selected from C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R₁₂)_mC(O)R₈, (CR₁₂R₁₂)_mC(S)R₈, (CR₁₂R₁₂)_mS(O)R₈, (CR₁₂R₁₂)_mS(O)₂R₈, (CR₁₂R₁₂)_mOR₉, (CR₁₂R₁₂)_mSR₉, (CR₁₂R₁₂)_mNR₁₀R₁₁, (CR₁₂R₁₂)_mC(=NR₂₄)R₂₂ and (CR₁₂R₁₂)_mR₁₃;

25 R₃ is selected from hydrogen, C₁-C₆alkyl, (CR₁₆R₁₆)_pNR₁₄R₁₅, (CR₁₆R₁₆)_pOR₁₇, (CR₁₆R₁₆)_pSR₁₇, (CR₁₆R₁₆)_phalo, (CR₁₆R₁₆)_pNO₂, (CR₁₆R₁₆)_nC(O)R₂₈, (CR₁₆R₁₆)_nC(=NR₂₄)R₂₂, (CR₁₆R₁₆)_nS(O)R₁₇, (CR₁₆R₁₆)_nS(O)₂R₁₇, (CR₁₆R₁₆)_nS(O)₃R₁₇ and (CR₁₆R₁₆)_pC(R₁₈)₃;

R_4 is selected from hydrogen, halogen C_1 - C_3 alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl and $(CR_{12}R_{12'})_n C(R_{18})_3$;

- 5 Each R_5 and $R_{5'}$ is independently selected from hydrogen, C_1 - C_3 alkyl, halo, OR_7 , SR_7 and $N(R_6)_2$;

Each R_6 is independently selected from hydrogen, C_1 - C_3 alkyl and OR_7 ;

- 10 Each R_7 is independently selected from hydrogen and C_1 - C_3 alkyl;

R_8 is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, OR_{19} , SR_{19} , $N(R_{20})_2$, $[NH-CH(R_{21})-C(O)]_q-OR_{29}$, [sugar] $_q$ and $(CR_{12}R_{12'})_t R_{13}$;

- 15 R_9 is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{12}R_{12'})_t R_{13}$, $C(O)R_{23}$, CO_2R_{23} , $C(S)R_{23}$, $C(S)OR_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q-R_{23}$ and [sugar] $_q$;

- 20 R_{10} and R_{11} are independently selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{12}R_{12'})_m R_{13}$, $C(O)R_{23}$, $C(S)R_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q-R_{23}$, -[sugar] $_q$ and $NHC(=NR_{25})-NH_2$;

Each R_{12} and $R_{12'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, OR_{24} , SR_{24} , halo, $N(R_{24})_2$, CO_2R_{24} , CN, NO_2 , aryl or heterocyclyl;

25

R_{13} is selected from OR_{25} , SR_{25} , halo, $N(R_{25})_2$, $C(O)R_{31}$, CN, $C(R_{18})_3$, aryl or heterocyclyl;

R_{14} and R_{15} are independently selected from hydrogen, C_1 - C_3 alkyl, OR_{17} , $(CR_{16}R_{16'})_p C(R_{18})_3$;

30

Each R_{16} and $R_{16'}$ is independently selected from hydrogen, C_1 - C_3 alkyl, halo, OR_{17} , SR_{17} and $N(R_{17})_2$;

Each R_{17} is independently selected from hydrogen and C_1 - C_3 alkyl;

Each R_{18} is independently selected from hydrogen and halo;

5

R_{19} and each R_{20} are independently selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, $(CR_{26}R_{26'})_tR_{27}$;

R_{21} is the characterising group of an amino acid;

10

R_{22} is selected from C_1 - C_6 alkyl, NH_2 , $NH(C_1$ - C_6 alkyl), $N(C_1$ - C_6 alkyl) $_2$, OR_{29} or SR_{29} ;

R_{23} is selected from hydrogen, C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{20} alkynyl, aryl $(CR_{26}R_{26'})_tR_{27}$;

15

Each R_{24} is independently selected from hydrogen and C_1 - C_6 alkyl;

Each R_{25} is independently selected from hydrogen, C_1 - C_6 alkyl, C_1 - C_3 alkoxy C_1 - C_3 alkyl, aryl and heterocyclyl;

20

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, OR_{29} , SR_{29} , halo, $N(R_{29})_2$, CO_2R_{29} , CN , NO_2 , aryl and heterocyclyl;

R_{27} is selected from hydrogen, OR_{30} , SR_{30} , halo, $N(R_{30})_2$, CO_2R_{30} , aryl and heterocyclyl;

25

R_{28} is selected from hydrogen, C_1 - C_6 alkyl, OR_{29} , SR_{29} or $N(R_{29})_2$;

Each R_{29} is independently selected from hydrogen and C_1 - C_3 alkyl;

30 Each R_{30} is independently selected from hydrogen, C_1 - C_3 alkyl, aryl and heterocyclyl;

R_{31} is selected from C_{1-3} alkyl, OH, C_{1-3} alkoxy, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

n is 0 or an integer from 1 to 3;

5 m is 0 or an integer from 1 to 20;

p is 0 or an integer from 1 to 6;

q is an integer from 1 to 5;

t is an integer from 1 to 10;

10 wherein alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

2. A method according to claim 1 wherein X is selected from the group consisting of -N(H)-, -N(C_{1-3} alkyl)-, -N(OH)-, -N(OC $_{1-3}$ alkyl)-, -O-, -S-, -CH₂-, -CH(OH)-, -CH(NH₂)-, -CH(C_{1-3} alkyl)-, -CH(halo)-, -CH(SH)-, -CH(OC $_{1-3}$ alkyl)-, -CH(SC $_{1-3}$ alkyl)-.

15

3. A method according to claim 1 wherein Y is selected from the group consisting of -NH-, -O-, -S-, -N(C_{1-3} alkyl)- or -CH₂-.

4. A method according to claim 1 wherein Z is selected from the group consisting of
20 -C(O)-, -C(S)-, -C(=NH)-, -C(=NC $_{1-3}$ alkyl)-, -C(=NOH)- or -C(=NOC $_{1-3}$ alkyl).

5. A method according to claim 1 wherein R_1 is selected from the group consisting of hydrogen, CH₃, OH, SH, NH₂, NHCH₃, F, Cl or Br.

25 6. A method according to claim 1 wherein R_2 is selected from the group consisting of C_{1-20} alkyl, C_{1-20} alkenyl, (CR₁₂R_{12'})_mheterocyclyl, (CR₁₂R_{12'})_maryl, (CR₁₂R_{12'})_mhalo, (CR₁₂R_{12'})_mOH, (CR₁₂R_{12'})_mOC $_{1-20}$ alkyl, (CR₁₂R_{12'})_mOC $_{2-20}$ alkenyl, (CR₁₂R_{12'})_mOC(O) C_{1-20} alkyl, (CR₁₂R_{12'})_mOC(O) C_{2-20} alkenyl, (CR₁₂R_{12'})_mOC(O)aryl, (CR₁₂R_{12'})_mO[C(O)CH(R₂₁)NH]_r-H, (CR₁₂R_{12'})_mO[sugar]_r, (CR₁₂R_{12'})_mNH₂,
30 (CR₁₂R_{12'})_mNHC $_{1-20}$ alkyl, (CR₁₂R_{12'})_mN(C_{1-20} alkyl)₂, (CR₁₂R_{12'})_mNHC $_{2-20}$ alkenyl, (CR₁₂R_{12'})_mN(C_{2-20} alkenyl)₂, (CR₁₂R_{12'})_mN(C_{1-20} alkyl)(C_{2-20} alkenyl), (CR₁₂R_{12'})_mNHC(O) C_{1-20} alkyl, (CR₁₂R_{12'})_mNHC(O) C_{2-20} alkenyl, (CR₁₂R_{12'})_mNHC(O)aryl,

$(\text{CR}_{12}\text{R}_{12'})_m\text{NH}[\text{C}(\text{O})\text{CH}(\text{R}_{21})\text{NH}]_r\text{H}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{NH}[\text{sugar}]_r$, $(\text{CR}_{12}\text{R}_{12'})_m\text{SO}_3\text{H}$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{SO}_3\text{C}_{1-20}\text{alkyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{SO}_3\text{C}_{2-20}\text{alkenyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{C}_{1-20}\text{alkyl}$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{C}_{2-20}\text{alkenyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{CO}_2\text{H}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{CO}_2\text{C}_{1-20}\text{alkyl}$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{CO}_2\text{C}_{2-20}\text{alkenyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{NHC}_{1-20}\text{alkyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{N}(\text{C}_{1-20}\text{alkyl})_2$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{NHC}_{2-20}\text{alkenyl}$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{N}(\text{C}_{2-20}\text{alkenyl})_2$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})\text{N}(\text{C}_{1-20}\text{alkyl})(\text{C}_{2-20}\text{alkenyl})$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})[\text{NHCH}(\text{R}_{21})\text{C}(\text{O})]_r\text{OH}$,
 $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})[\text{NHCH}(\text{R}_{21})\text{C}(\text{O})]_r\text{OCH}_3$, $(\text{CR}_{12}\text{R}_{12'})_m\text{C}(\text{O})[\text{sugar}]_r$, $(\text{CR}_{12}\text{R}_{12'})_m\text{SC}_{1-6}\text{alkyl}$, $\text{C}(=\text{N})\text{NHC}_{1-6}\text{alkyl}$; wherein each R_{12} and $\text{R}_{12'}$ is independently selected from
hydrogen, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$, halogen, OH, hydroxy $\text{C}_{1-6}\text{alkyl}$, $\text{OC}_{1-6}\text{alkyl}$,
 CO_2H , $\text{CO}_2\text{C}_{1-3}\text{alkyl}$, NH_2 , $\text{NHC}_{1-3}\text{alkyl}$, $\text{N}(\text{C}_{1-3}\text{alkyl})_2$, CN, NO_2 , aryl or heterocyclyl; R_{21}
is the characterising group of an amino acid, m is 0 or an integer from 1 to 20 and r is an
integer from 1 to 5.

7. A method according to claim 1 wherein R_3 is selected from the group consisting of
hydrogen, halogen, $\text{C}_1\text{-C}_6\text{alkyl}$, $-(\text{CH}_2)_n\text{NH}_2$, $-(\text{CH}_2)_n\text{NO}_2$, $-(\text{CH}_2)_n\text{OH}$, $-(\text{CH}_2)_n\text{CF}_3$ or
 $-(\text{CH}_2)_n\text{SH}$ wherein n is as defined in claim 1.

8. A method according to claim 1 wherein R_4 is selected from the group consisting of
hydrogen, methyl, ethyl, $-\text{CH}_2=\text{CH}_2$, CH_2CF_3 , fluoro, chloro or bromo.

9. A method according to claim 1 wherein at least one of R_5 and $\text{R}_{5'}$ in each $(\text{CR}_5\text{R}_{5'})$
is hydrogen.

10. A method according to claim 1 wherein at least one of R_{12} and $\text{R}_{12'}$ in each
 $(\text{CR}_{12}\text{R}_{12'})$ is hydrogen.

11. A method according to claim 1 wherein at least one of R_{16} and $\text{R}_{16'}$ in each
 $(\text{CR}_{16}\text{R}_{16'})$ is hydrogen.

12. A method according to claim 1 wherein at least one of R_{26} and $\text{R}_{26'}$ in each
 $(\text{CR}_{26}\text{R}_{26'})$ is hydrogen.

13. A method according to claim 1 wherein

X is selected from the group consisting of -O-, -S-, -C(R₅)₂- or -N(R₆)-;

Y is selected from the group consisting of -N(R₇)-, -O-, -S-, or -C(R₇)₂-;

Z is selected from the group consisting of -C(O)-, -C(S)-, -S(O)- or -C(=NR₆);

10 R₁ is selected from the group consisting of hydrogen, CH₃, OH, SH, NH₂, NHCH₃, F, Cl or Br;

R₂ is selected from the group consisting of C₁-C₂₀alkyl, C₂-C₂₀alkenyl, C₂-C₂₀alkynyl, (CR₁₂R_{12'})_mC(O)R₈, -(CR₁₂R_{12'})_mC(S)R₈, -(CR₁₂R_{12'})_mS(O)R₈, -(CR₁₂R_{12'})_mS(O)₂R₈, -
15 (CR₁₂R_{12'})_mOR₉, -(CR₁₂R_{12'})_mSR₉, -(CR₁₂R_{12'})_mNR₁₀R₁₁, (CR₁₂R_{12'})_mC(=NR₂₄)R₂₂ or (CR₁₂R_{12'})_mR₁₃ where m, R₇, R₈, R₉, R₁₀, R₁₁, R₁₂, R_{12'}, R₁₃, R₂₂ and R₂₄ are as defined in claim 1;

R₃ is hydrogen, halogen, C₁₋₆alkyl, -(CH₂)_nNH₂, -(CH₂)_nNO₂, -(CH₂)_n-OH, -(CH₂)_nCF₃ or -
20 (CH₂)_nSH where n is as defined in claim 1; and

R₄ is hydrogen, halogen, methyl, ethyl, CH₂CF₃ or -CH₂=CH₂.

14. A method according to claim 1 wherein

25 X is -N(R₆)-;

Y is -N(R₇)- or -C(R₇)₂-;

Z is -C(O)-, -C(S)-, -S(O)- or -C(=NH);

30

R₁ is hydrogen, CH₃, NH₂, NHCH₃, F, Cl or Br;

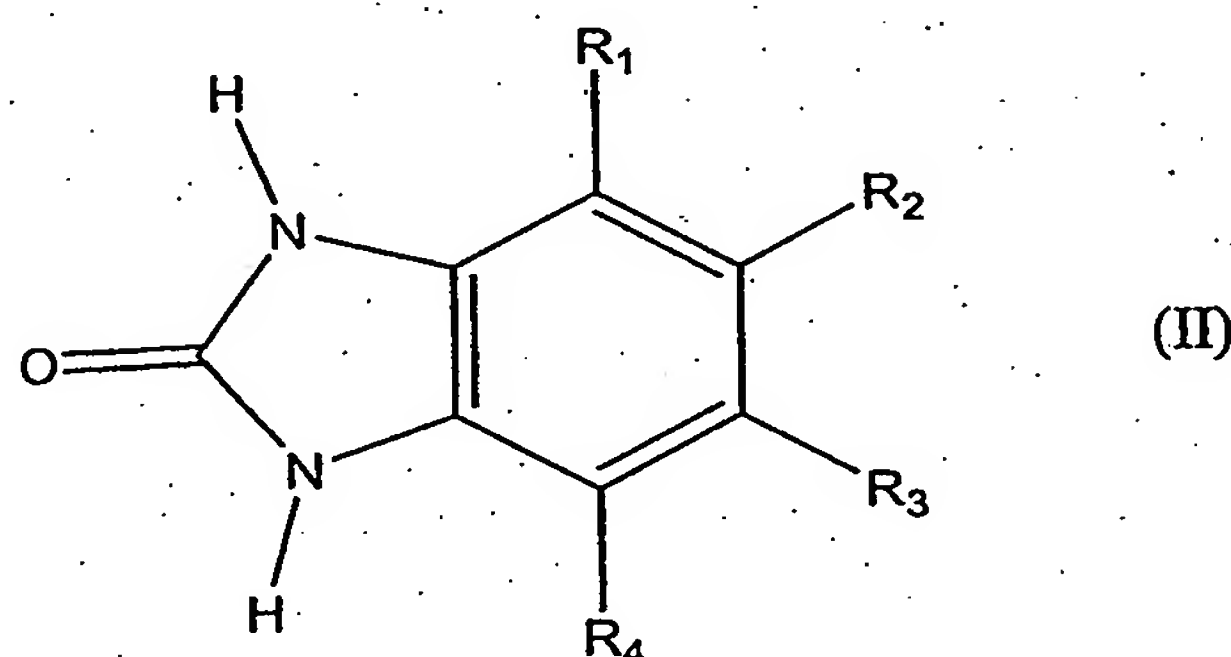
R_2 is as defined in claim 1;

R_3 is hydrogen, halogen, C_{1-3} alkyl, $(CH_2)_nNH_2$, $-(CH_2)_nNO_2$, $(CH_2)_nOH$ or $(CH_2)_nCF_3$

5 where n is defined in claim 1; and

R_4 is hydrogen, halogen, methyl, ethyl, CH_2CF_3 or $-CH_2=CH_2$.

15 A method according to claim 1 wherein the compound of formula (I) is a benzimidazole compounds having the formula (II):



wherein

15

R_1 is hydrogen, CH_3 , $NHCH_3$, F, Cl or Br;

R_2 is as defined in claim 1;

20

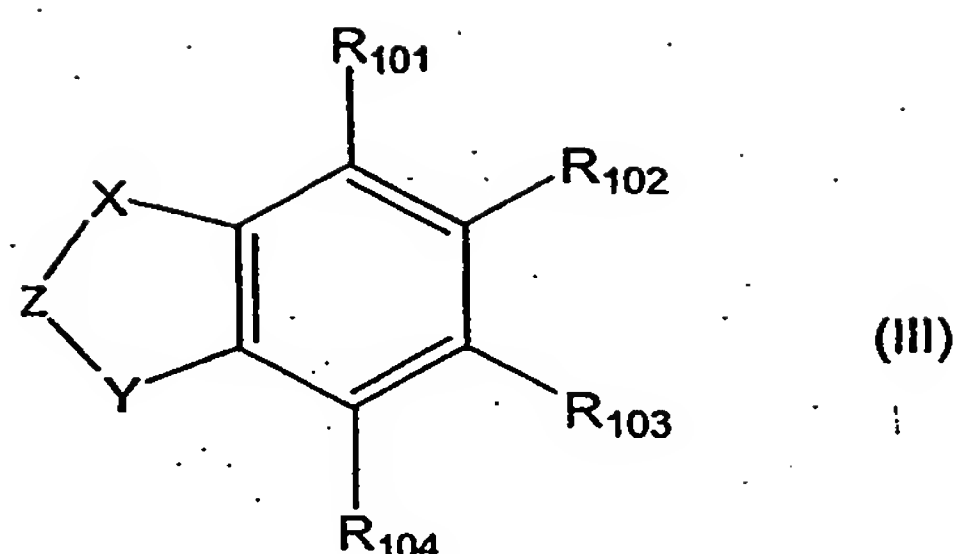
R_3 is hydrogen, halogen, C_{1-3} alkyl, $(CH_2)_nNH_2$, $-(CH_2)_nNO_2$, $(CH_2)_nOH$, $CH_2C(O)CH_3$, or $(CH_2)_nCF_3$ where n is as defined in claim 1; and

R_4 is hydrogen, F, Cl or Br, methyl, ethyl, CH_2CF_3 or $-CH_2=CH_2$.

25

16. A method according to claim 1 wherein the compound of formula (I) is a compound of formula (III):

121



wherein

5

X is -O-, -NH- or -CH₂-;

Y is -NH-, -O-, -S- or -CH₂-;

10

Z is -C(O)-, -C(S)- or -S(O)-;

R₁₀₁ is selected from hydrogen, C₁₋₃alkyl, OH, SH, NH₂, NHC₁₋₃alkyl, F, Cl or Br;

R₁₀₂ is selected from C₁₋₂₀alkyl, C₂₋₂₀alkenyl, CO₂H, CO₂R₁₀₅, -NH₂, F, Cl, Br, (CH₂)_wR₁₀₆,
 15 C(O)N(R₁₀₇)₂, C(=N)NHC₁₋₆alkyl, SO₂C₁₋₆alkyl, C(O)[NHCH(R₁₀₈)C(O)]_q-OR₁₀₉,
 C(O)sugar, CONH(CH₂)_naryl, NHC(O)(CH₂)_nSheterocyclyl, C(O)SC₁₋₆alkyl,
 C(O)(CH₂)_nCO₂H, SO₂OC₁₋₁₀alkyl, and SO₂NHC₁₋₁₀alkyl;

R₁₀₃ is selected from hydrogen, F, Cl, Br, C₁₋₆alkyl, -(CH₂)_nNH₂, -(CH₂)_nNO₂, -(CH₂)_n-OH,
 20 -(CH₂)_n-CF₃, -(CH₂)_nC(O)C₁₋₃alkyl or -(CH₂)_n-SH;

R₁₀₄ is selected from hydrogen, methyl, ethyl, CH₂C(R₁₁₀)₃, C(R₁₁₀)₃, -CH₂=CH₂, fluoro,
 chloro or bromo;

25

R₁₀₅ is selected from hydrogen, C₁₋₂₀alkyl, C₂₋₂₀alkenyl or (CH₂)_tOC₁₋₃alkyl;

R₁₀₆ is selected from SH, SC₁₋₆alkyl, OH, OC₁₋₆alkyl, sugar, CO₂H, NH₂, heterocyclyl or

aryl;

Each R_{107} is independently selected from hydrogen, C_{1-20} alkyl, C_{2-20} alkenyl, $(CH_2)_t$ aryl and $(CH_2)_t$ heterocyclyl;

5

R_{108} is the characterising group of an amino acid;

R_{109} is hydrogen, C_{1-3} alkyl;

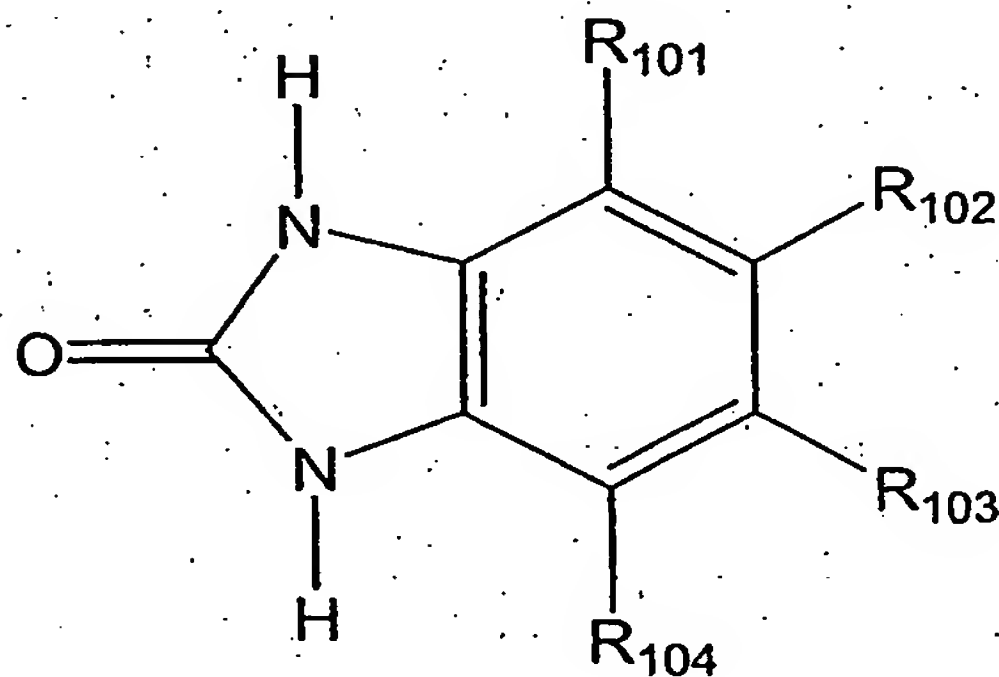
10

Each R_{110} is independently selected from hydrogen and halo; and

n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6; t is an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

15

17. A method according to claim 1 wherein the compound of formula 1 is a compound of formula (IV):



(IV)

20

wherein

R_{101} is selected from hydrogen, CH_3 , OH, SH, NH_2 , $NHCH_3$, F, Cl or Br;

R_{102} is selected from C_{1-20} alkyl, C_{2-20} alkenyl, CO_2H , CO_2R_{105} , $-NH_2$, F, Cl, Br, $(CH_2)_wR_{106}$,

25

$C(O)N(R_{107})_2$, $C(=N)NHC_{1-6}$ alkyl, SO_2C_{1-6} alkyl, $C(O)[NHCH(R_{108})C(O)]_q-OR_{109}$, $C(O)sugar$, $CONH(CH_2)_n$ aryl, $NHC(O)(CH_2)_n$ Sheterocyclyl, $C(O)SC_{1-6}$ alkyl,

$C(O)(CH_2)_nCO_2H$, $SO_2OC_{1-10}alkyl$, and $SO_2NHC_{1-10}alkyl$;

R_{103} is selected from hydrogen, F, Cl, Br, $C_{1-6}alkyl$, $(CH_2)_nNH_2$, $-(CH_2)_nNO_2$, $-(CH_2)_n-OH$, $-(CH_2)_n-CF_3$, $CH_2C(O)CH_3$ or $-(CH_2)_n-SH$;

R_{104} is selected from hydrogen, methyl, ethyl, CH_2CF_3 , $-CH_2=CH_2$ fluoro, chloro or bromo;

R_{105} is selected from hydrogen, $C_{1-10}alkyl$, $C_{2-10}alkenyl$, $(CH_2)_tOC_{1-3}alkyl$;

R_{106} is selected from SH, $SC_{1-6}alkyl$, OH, $OC_{1-6}alkyl$, sugar, CO_2H , NH_2 , heterocyclyl or aryl;

Each R_{107} is independently selected from hydrogen, $C_{1-10}alkyl$, $C_{2-10}alkenyl$, $(CH_2)_taryl$ and $(CH_2)_theterocyclyl$;

R_{108} is the characterising group of an amino acid;

R_{109} is hydrogen, $C_{1-3}alkyl$;

Each R_{110} is independently selected from hydrogen and halo; and

n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6, t is an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

18. A method according to claim 1 wherein the compound of formula 1 is selected from the group consisting of:

benzimidazole-2-one-5-n-pentanoate,

5-[2-(1-oxy-2-hydroxyethyl)ethyl]benzimidazol-2-one-5-carboxylate,

benzimidazole-2-one-5-methanoate,

benzimidazole-2-one-5-ethanoate,
 3,4,5-tris(acetyloxy)-6-[(acetyloxy)methyl]tetrahydro-2H-pyran-2-yl-
 benzimidazole-2-one-5-carboxylate,
 5-bromo-6-methylbenzimidazol-2-one,
 5-hydroxy-6-methylbenzimidazol-2-one,
 5-dodecanylbzenzimidazol-2-one,
 4,5,7-tribromo-6-methylbenzimidazol-2-one,
 4,5,6,7-tetrabromobenzimidazol-2-one,
 5-methyl-6-nitrobenzimidazol-2-one,
 5-amino-6-methylbenzimidazol-2-one,
 N-(6-methylbenzimidazol-5-yl)-2-pyrimidin-2-yl-sulfanyl-acetamide,
 pentyl-benzimidazol-2-one-5-carbothioate,
 5-(benzimidazol-2(3H)-one-6-yl)-5-oxopentanoic acid,
 2(3H)-benzimidazolone-5-sulfonic acid pentyl ester,
 2(3H)-benzimidazolone-5-sulfonic acid pentyl amide,
 N-butyl-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboximidamide,
 5-heptanoylbenzofuran-2(3H)-one,
 methyl 3-hydroxy-2-[[[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}propanoate,
 3-hydroxy-2-[[[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}propanoic acid,
 methyl 2-[[[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenyl propanoate,
 2-[[[(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenyl propanoic acid, and
 N-(3,4-dihydroxyphenethyl)-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxamide.

19. A method of treating, preventing or diagnosing a disease or condition wherein MIF
 cytokine or biological activity is implicated comprising the administration of a treatment,
 prevention or diagnostic effective amount of a compound of formula (I) as defined in claim
 1 or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

20. A method according to claim 19 wherein the disease or condition is selected from autoimmune diseases, solid or haemopoietic tumours and chronic or acute inflammatory diseases.

5

21. A method according to claim 19 wherein the disease or condition is selected from the group consisting of Rheumatic diseases, spondyloarthropathies, crystal arthropathies, Lyme disease, connective tissue diseases, vasculitides, glomerulonephritis, interstitial nephritis, inflammatory bowel disease, peptic ulceration, gastritis, oesophagitis, liver
10 disease, autoimmune diseases, pulmonary diseases, cancers whether primary or metastatic, atherosclerosis, disorders of the hypothalamic-pituitary-adrenal axis, brain disorders, corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis, endometrial function, psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic
15 inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases, atopic dermatitis, UV(B)-induced dermal cell activation, malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing.

20

22. A method according to claim 21 wherein the disease or condition is selected from the group consisting of rheumatoid arthritis, osteoarthritis, psoriatic arthritis, ankylosing spondylitis, reactive arthritis, Reiter's syndrome, gout, pseudogout, calcium pyrophosphate deposition disease, systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome, polyarteritis nodosa, Wegener's granulomatosis,
25 Churg-Strauss syndrome, ulcerative colitis, Crohn's disease, cirrhosis, hepatitis, diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis, diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach
30 cancer, leukemia, cervical cancer and metastatic cancer, ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease, Alzheimer's disease, multiple sclerosis, diabetic retinopathy, parturition, endometriosis, osteoporosis, Paget's disease,

sunburn and skin cancer.

23. A method of claim 19 wherein the subject is a human subject.

5 24. A pharmaceutical composition comprising a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or prodrug thereof and a pharmaceutically acceptable carrier, diluent or excipient

10 25. A pharmaceutical composition according to claim 24 further comprising a glucocorticoid.

26. A method of treating or preventing a disease or condition wherein MIF cytokine or biological activity is implicated comprising:

15 administering to a mammal a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or prodrug thereof and a second therapeutic agent.

27. A method according to claim 26 wherein the second therapeutic agent is a glucocorticoid.

20

28. A method of prophylaxis or treatment of a disease or condition for which treatment with a glucocorticoid is indicated, said method comprising:

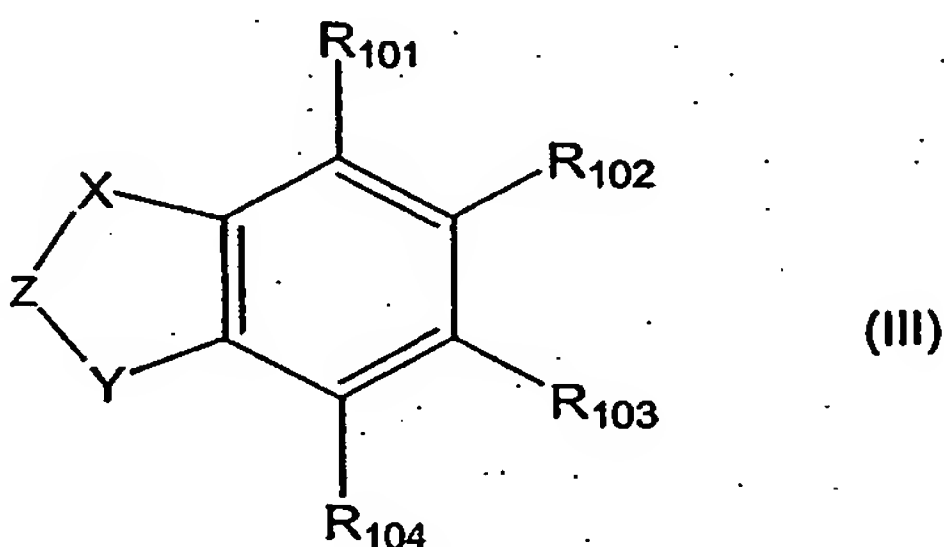
25 administering to a mammal a glucocorticoid and a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or prodrug thereof.

29. A method of treating a steroid-resistant disease or condition comprising:

30 administering to a mammal a glucocorticoid and a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or prodrug thereof.

30. A method of enhancing the effect of a glucocorticoid in mammals comprising administering a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or prodrug thereof simultaneously, separately or sequentially with said glucocorticoid.

31. A compound of formula (III) or a pharmaceutically acceptable salt or prodrug thereof:



wherein

X is -O-, -NH- or -CH₂-;

Y is -NH-, -O-, -S- or -CH₂-;

Z is -C(O)-, -C(S)- or -S(O)-;

R₁₀₁ is selected from hydrogen, C₁₋₃alkyl, OH, SH, NH₂, NHC₁₋₃alkyl, F, Cl or Br;

R₁₀₂ is selected from C₁₋₂₀alkyl, C₂₋₂₀alkenyl, CO₂H, F, Cl, Br, CO₂R₁₀₅, (CH₂)_wR₁₀₆, C(O)N(R₁₀₇)₂, C(=N)NHC₁₋₆alkyl, SO₂C₁₋₆alkyl, C(O)[NHCH(R₁₀₈)C(O)]_q-OR₁₀₉, NH₂, C(O)sugar, CONH(CH₂)_naryl, NHC(O)(CH₂)_nSheterocyclyl, C(O)SC₁₋₆alkyl, C(O)(CH₂)_nCO₂H, SO₂OC₁₋₁₀alkyl and SO₂NHC₁₋₁₀alkyl;

R₁₀₃ is selected from hydrogen, F, Cl, Br, C₁₋₆alkyl, -(CH₂)_nNH₂, -(CH₂)_nNO₂, -(CH₂)_n-OH, -(CH₂)_n-CF₃, -(CH₂)_nC(O)C₁₋₃alkyl or -(CH₂)_n-SH;

R_{104} is selected from hydrogen, methyl, ethyl, $\text{CH}_2\text{C}(\text{R}_{110})_3$, $\text{C}(\text{R}_{110})_3$, $-\text{CH}_2=\text{CH}_2$, fluoro, chloro or bromo;

5 R_{105} is selected from hydrogen, C_{1-20} alkyl, C_{2-20} alkenyl or $(\text{CH}_2)_t\text{OC}_{1-3}$ alkyl;

R_{106} is selected from SH, SC_{1-6} alkyl, OH, OC_{1-6} alkyl, sugar, CO_2H , NH_2 , heterocyclyl or aryl;

10 Each R_{107} is independently selected from hydrogen, C_{1-20} alkyl, C_{2-20} alkenyl, $(\text{CH}_2)_t$ aryl and $(\text{CH}_2)_t$ heterocyclyl;

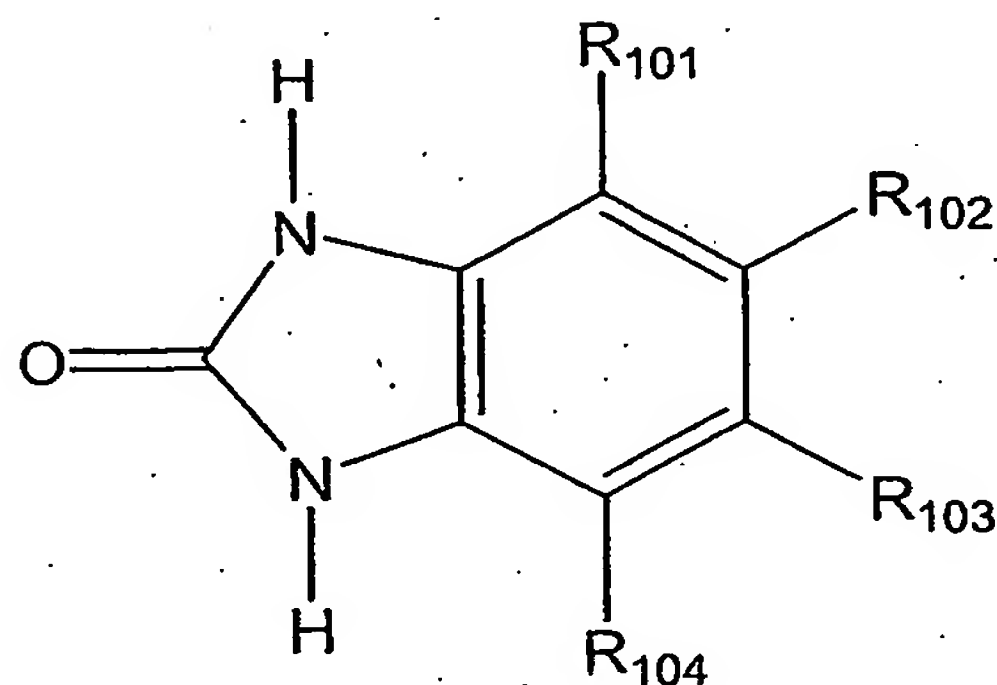
R_{108} is the characterising group of an amino acid;

15 R_{109} is hydrogen, C_{1-3} alkyl;

Each R_{110} is independently selected from hydrogen and halo; and

20 n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6; t is an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

32. A compound of formula (IV) or a pharmaceutically acceptable salt or prodrug thereof;



(IV)

wherein

R_{101} is selected from hydrogen, CH_3 , OH, SH, NH_2 , $NHCH_3$, F, Cl or Br;

5 R_{102} is selected from C_{1-20} alkyl, C_{2-20} alkenyl, CO_2H , F, Cl, Br, CO_2R_{105} , $(CH_2)_wR_{106}$, $C(O)N(R_{107})_2$, $C(=N)NHC_{1-6}$ alkyl, SO_2C_{1-6} alkyl, $C(O)[NHCH(R_{108})C(O)]_q-OR_{109}$, NH_2 , $C(O)sugar$, $CONH(CH_2)_naryl$, $NHC(O)(CH_2)_nSheterocyclyl$, $C(O)SC_{1-6}$ alkyl, $C(O)(CH_2)_nCO_2H$, SO_2OC_{1-10} alkyl and SO_2NHC_{1-10} alkyl.

10 R_{103} is selected from hydrogen, F, Cl, Br, C_{1-6} alkyl, $(CH_2)_nNH_2$, $-(CH_2)_nNO_2$, $-(CH_2)_n-OH$, $-(CH_2)_n-CF_3$, $CH_2C(O)CH_3$ or $-(CH_2)_n-SH$;

R_{104} is selected from hydrogen, methyl, ethyl, CH_2CF_3 , $-CH_2=CH_2$ fluoro, chloro or bromo;

15

R_{105} is selected from hydrogen, C_{1-10} alkyl, C_{2-10} alkenyl, $(CH_2)_tOC_{1-3}$ alkyl;

R_{106} is selected from SH, SC_{1-6} alkyl, OH, OC_{1-6} alkyl, sugar, CO_2H , NH_2 , heterocyclyl or aryl;

20

Each R_{107} is independently selected from hydrogen, C_{1-10} alkyl, C_{2-10} alkenyl, $(CH_2)_taryl$ and $(CH_2)_theterocyclyl$;

R_{108} is the characterising group of an amino acid;

25

R_{109} is hydrogen, C_{1-3} alkyl;

Each R_{110} is independently selected from hydrogen and halo; and

30

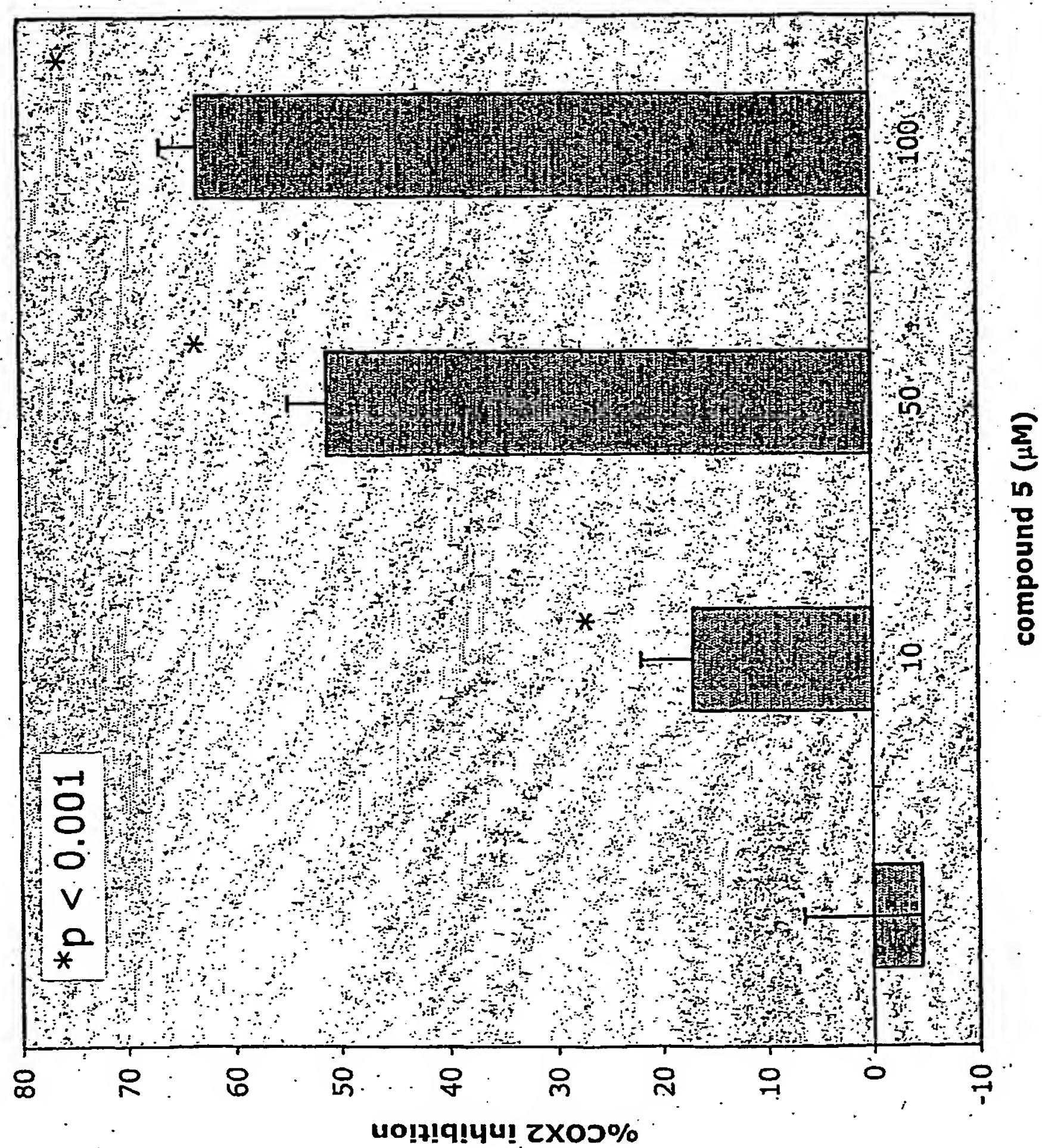
n is 0 or an integer from 1 to 3, q is an integer from 1 to 5, w is an integer from 1 to 6, t is an integer from 1 to 10; wherein each alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted.

33. A compound according to claim 32 wherein R_{101} is hydrogen, F, Cl or Br.
34. A compound according to claim 32 wherein R_{102} is C_{1-20} alkyl, halogen, NH_2 ,
 5 CO_2H , CO_2C_{1-10} alkyl, $C(O)$ sugar, $CO_2(CH_2)_nOC_{1-6}$ alkyl, $CONHC_{1-10}$ alkyl,
 $CONH(CH_2)_n$ aryl, $CO[NHCH(R_{107})CO]-OH$, $CO[NHCH(R_{107})CO]OC_{1-3}$ alkyl,
 $NHC(O)(CH_2)_n$ Sheterocyclyl, $C(O)SC_{1-6}$ alkyl, $C(O)(CH_2)_nCO_2H$, SO_2OC_{1-10} alkyl,
 SO_2NHC_{1-10} alkyl or $C(=NH)NHC_{1-6}$ alkyl.
- 10 35. A compound according to claim 32 wherein R_{103} is hydrogen, halogen, C_{1-3} alkyl,
 $(CH_2)_nNH_2$, $(CH_2)_nNO_2$, $(CH_2)_nNH_2$, $(CH_2)_nOH$ or $(CH_2)_nCF_3$.
36. A compound according to claim 32 wherein R_{104} is hydrogen, F, Cl or Br.
- 15 37. A compound according to claim 32 wherein R_{107} is the characterising group from
serine (CH_2OH) or phenylalanine (CH_2Ph).
38. A compound of formula (I) selected from the group consisting of:
 benzimidazole-2-one-5-n-pentanoate,
 20 5-[2-(1-oxy-2-hydroxyethyl)ethyl]benzimidazol-2-one-5-carboxylate,
 benzimidazole-2-one-5-methanoate,
 benzimidazole-2-one-5-ethanoate,
 3,4,5-tris(acetyloxy)-6-[(acetyloxy)methyl]tetrahydro-2H-pyran-2-yl-
 benzimidazole-2-one-5-carboxylate,
 25 5-bromo-6-methylbenzimidazol-2-one,
 5-hydroxy-6-methylbenzimidazol-2-one,
 5-dodecanylbzenzoimidazol-2-one,
 4,5,7-tribromo-6-methylbenzimidazol-2-one,
 4,5,6,7-tetrabromobenzimidazol-2-one,
 30 5-methyl-6-nitrobenzimidazol-2-one,
 5-amino-6-methylbenzimidazol-2-one,

- N-(6-methylbenzimidazol-5-yl)-2-pyrimidin-2-yl-sulfanyl-acetamide,
pentyl-benzimidazol-2-one-5-carbothioate,
5-(benzimidazol-2(3H)-one-6-yl)-5-oxopentanoic acid,
2(3H)-benzimidazolone-5-sulfonic acid pentyl ester,
5 2(3H)-benzimidazolone-5-sulfonic acid pentyl amide,
N-butyl-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboximidamide,
5-heptanoylbenzofuran-2(3H)-one,
methyl 3-hydroxy-2-{{(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-
yl)carbonyl]amino}propanoate,
10 3-hydroxy-2-{{(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-
yl)carbonyl]amino}propanoic acid,
methyl 2-{{(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-
phenyl propanoate,
2-{{(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)carbonyl]amino}-3-phenyl
15 propanoic acid, and
N-(3,4-dihydroxyphenethyl)-2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-
carboxamide.

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Figure 1



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Figure 2

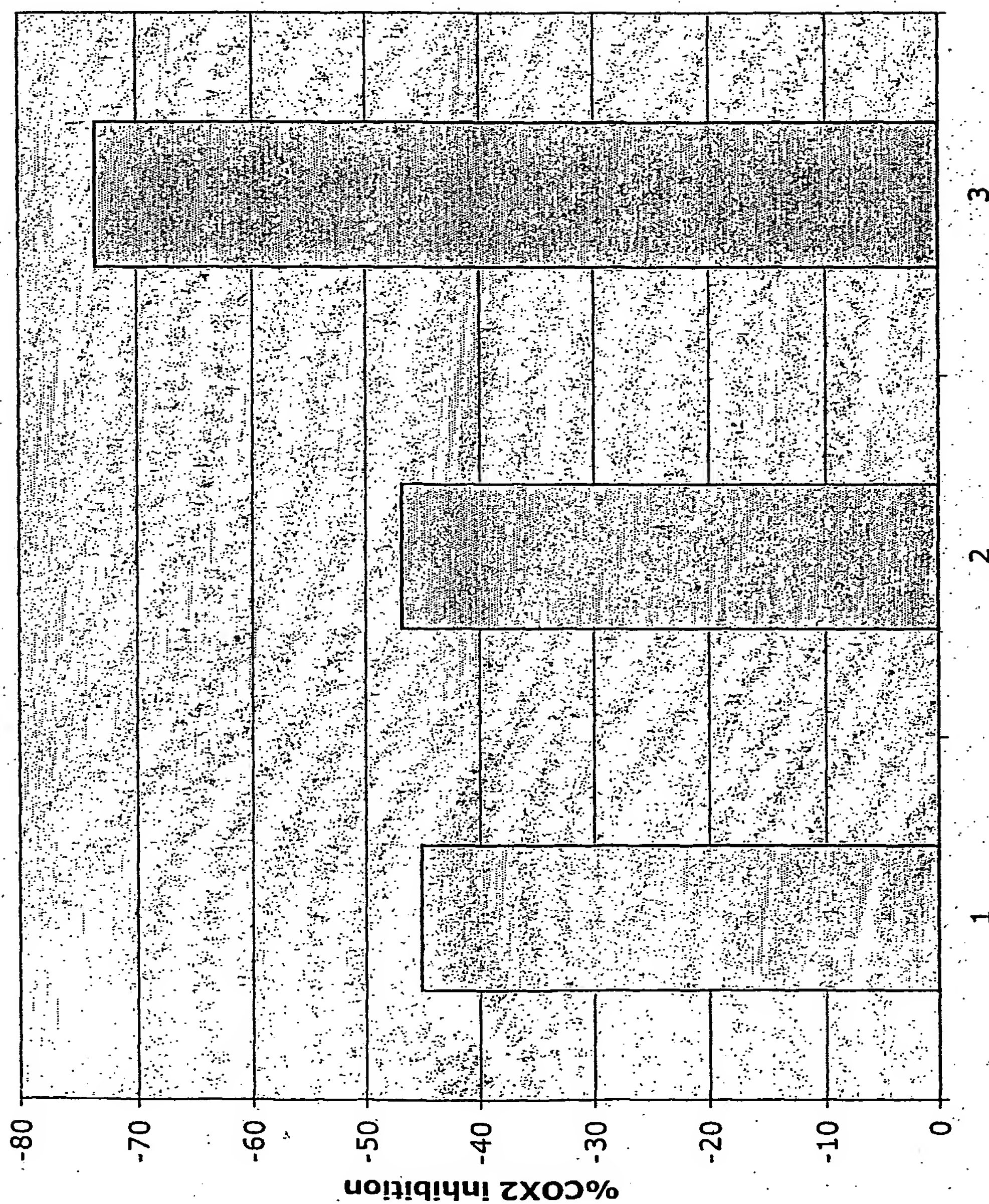
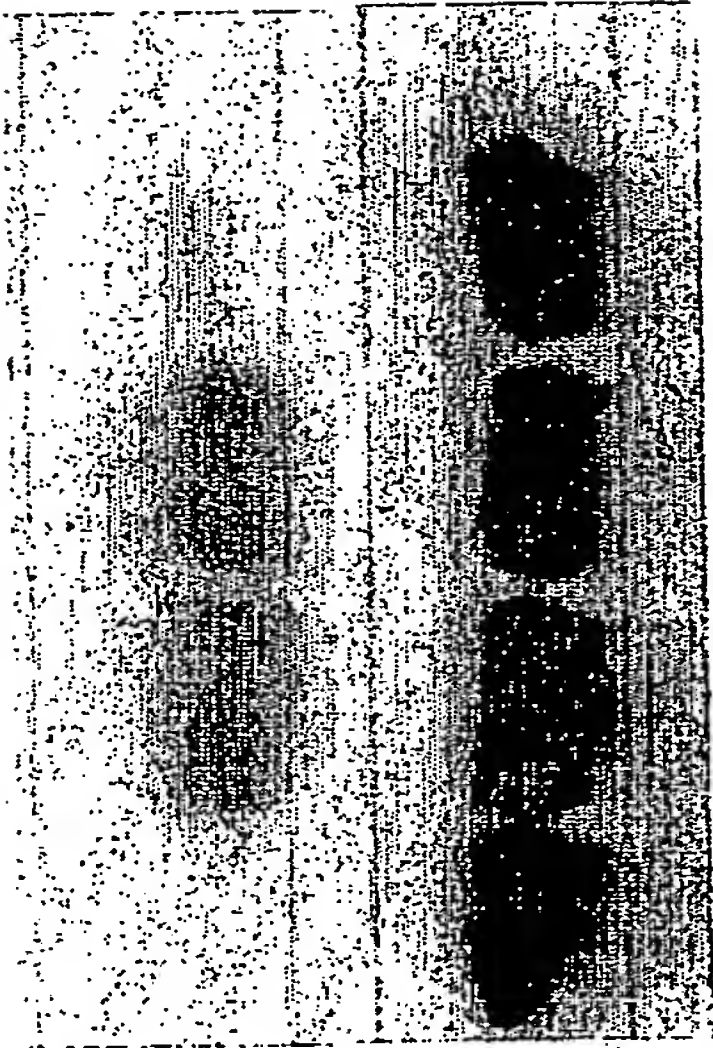


Figure 3



Phospho-ERK

Total ERK

1 2 3 4

*IL-1: Interleukin 1 (0.1 ng/ml)
MIF-a: benzimidazole-2-one-5-pentanoate (50 μ M)
DEX: dexamethasone (10^{-9} M)

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Figure 4

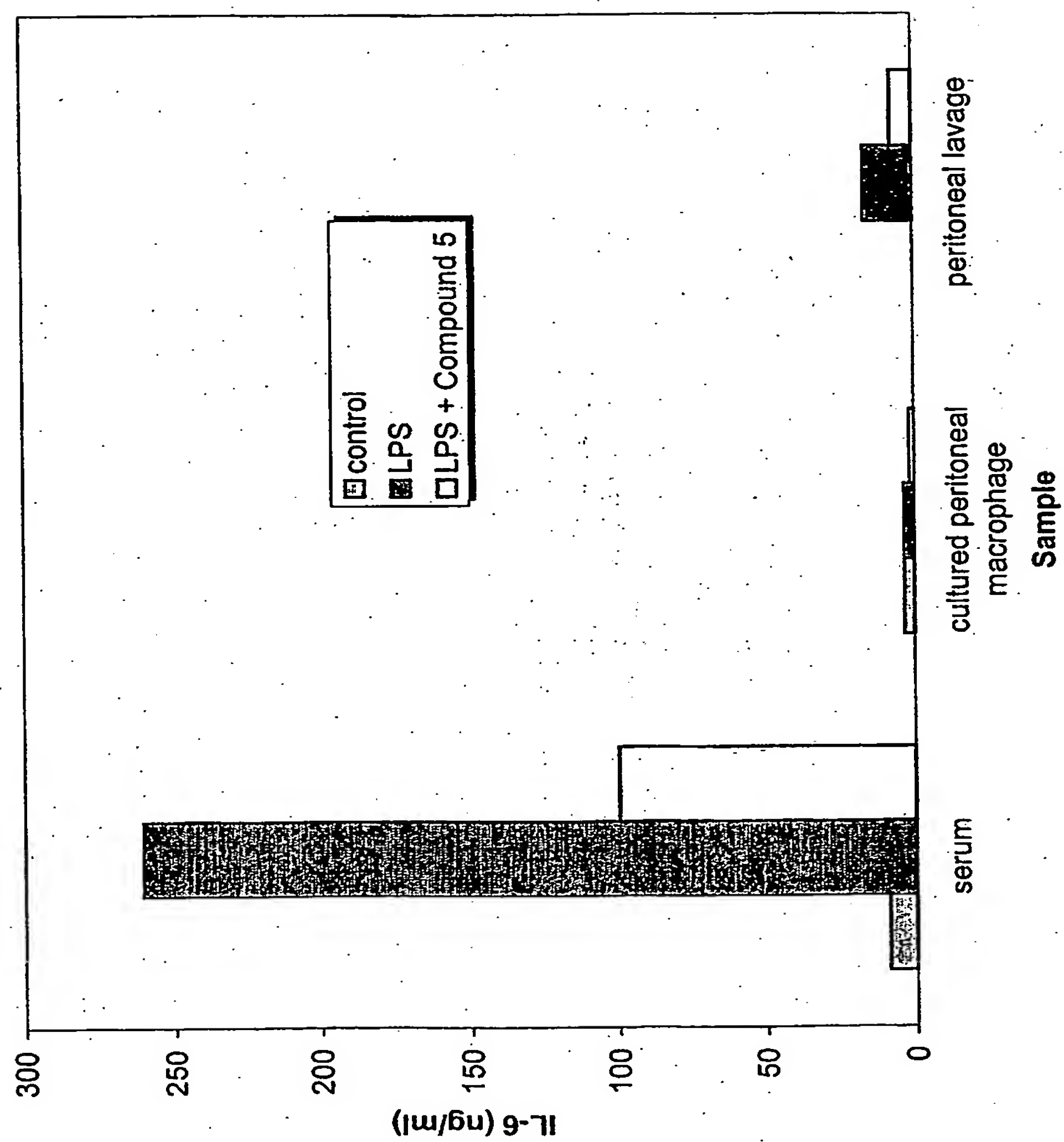


Figure 5

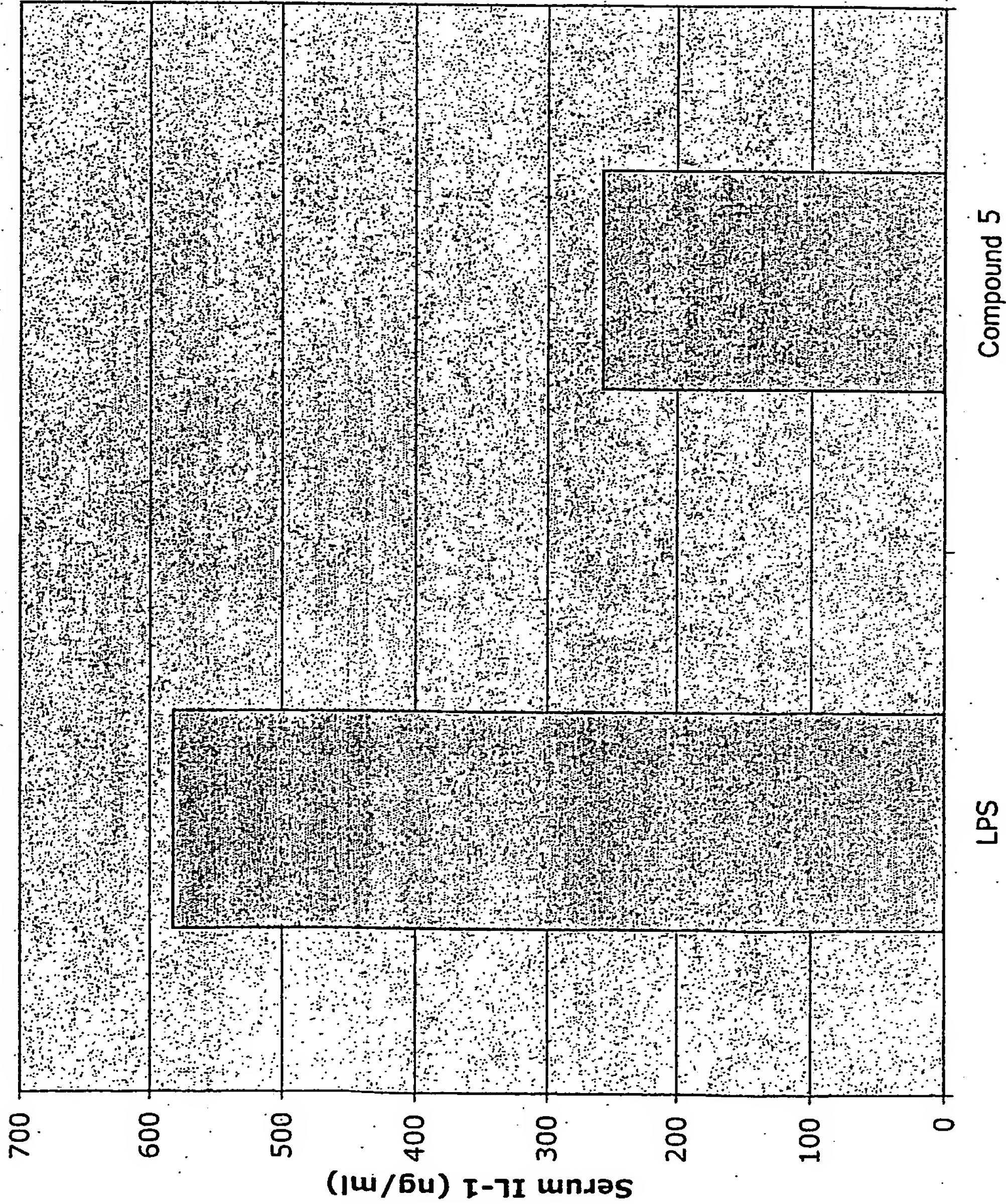
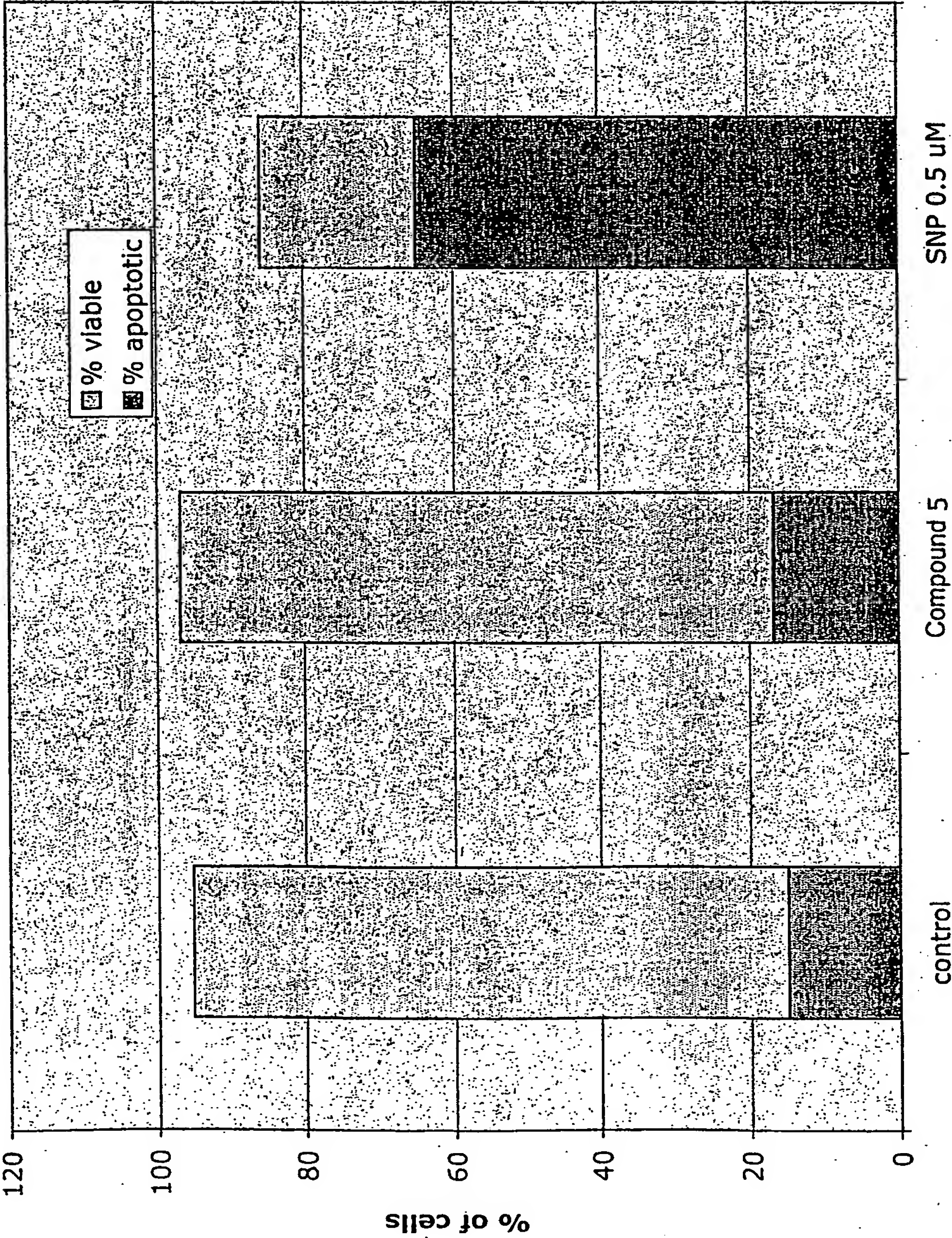
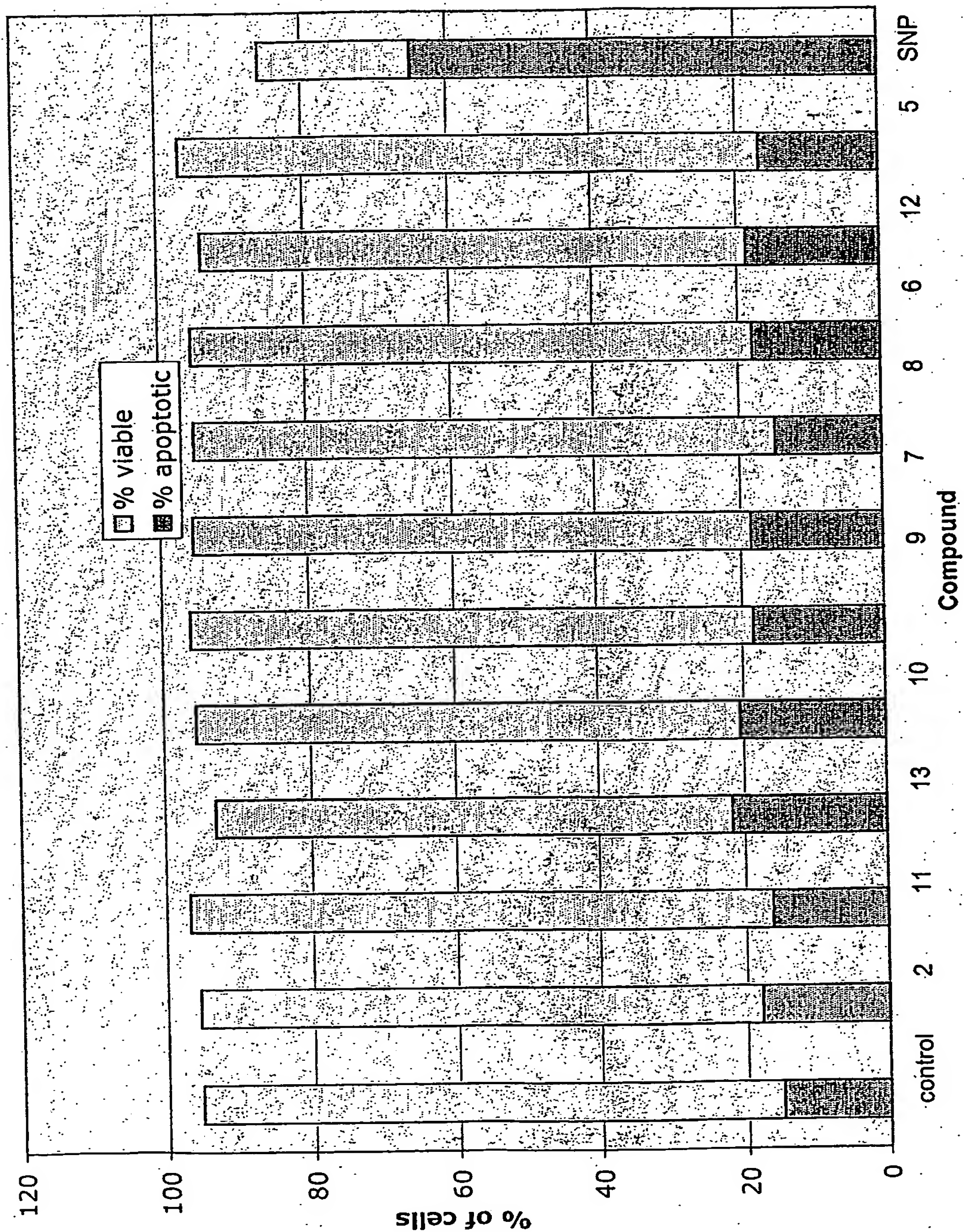


Figure 6



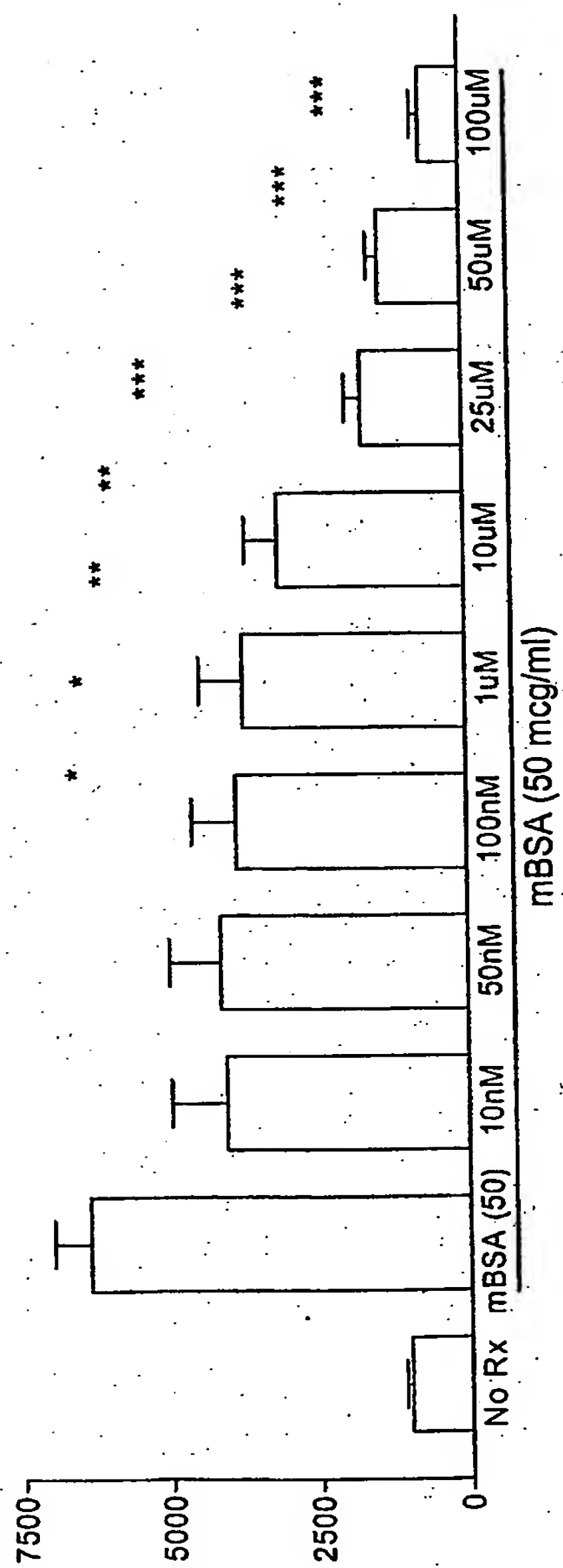
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Figure 7



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Figure 8



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Figure 9

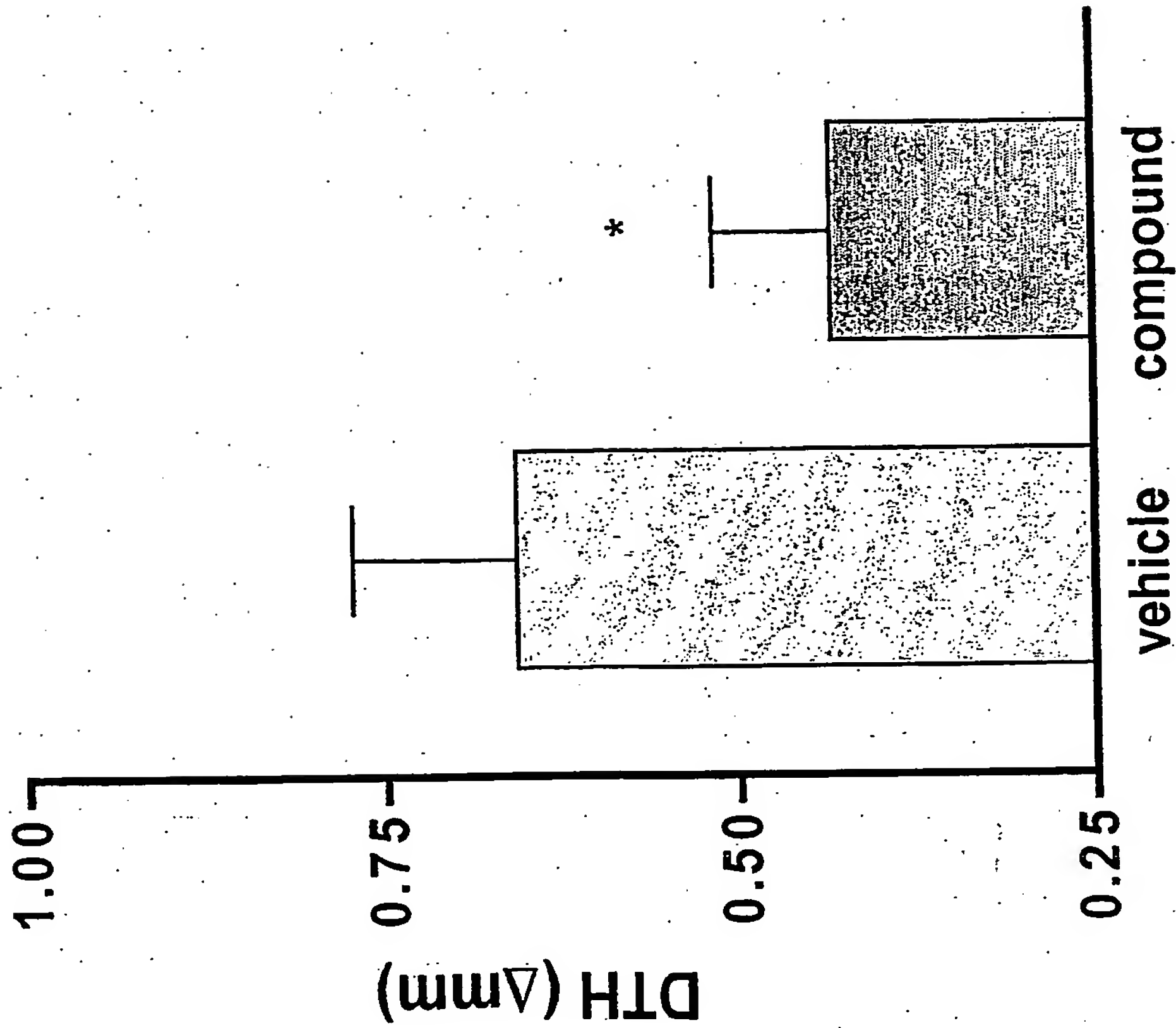
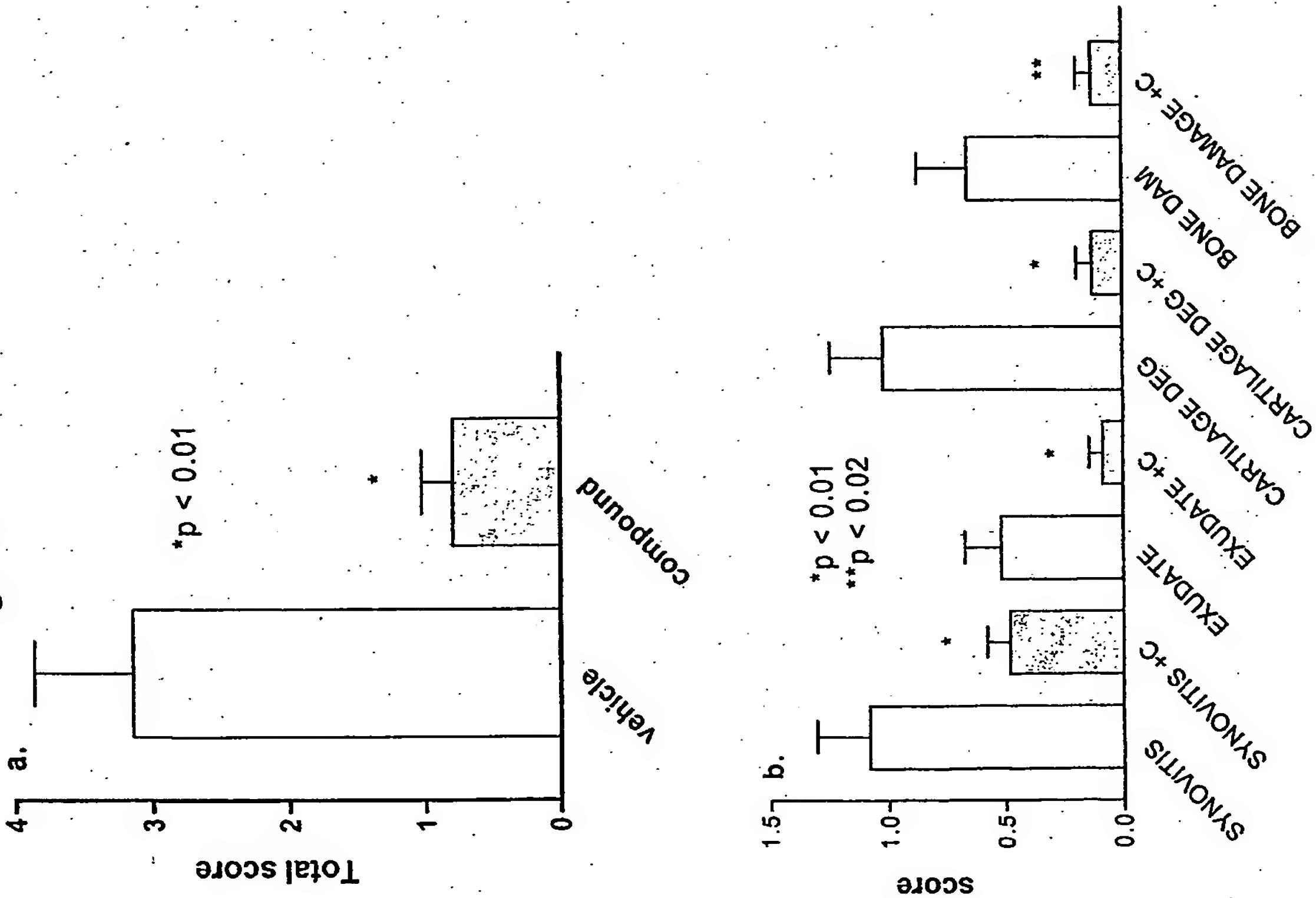


Figure 10.



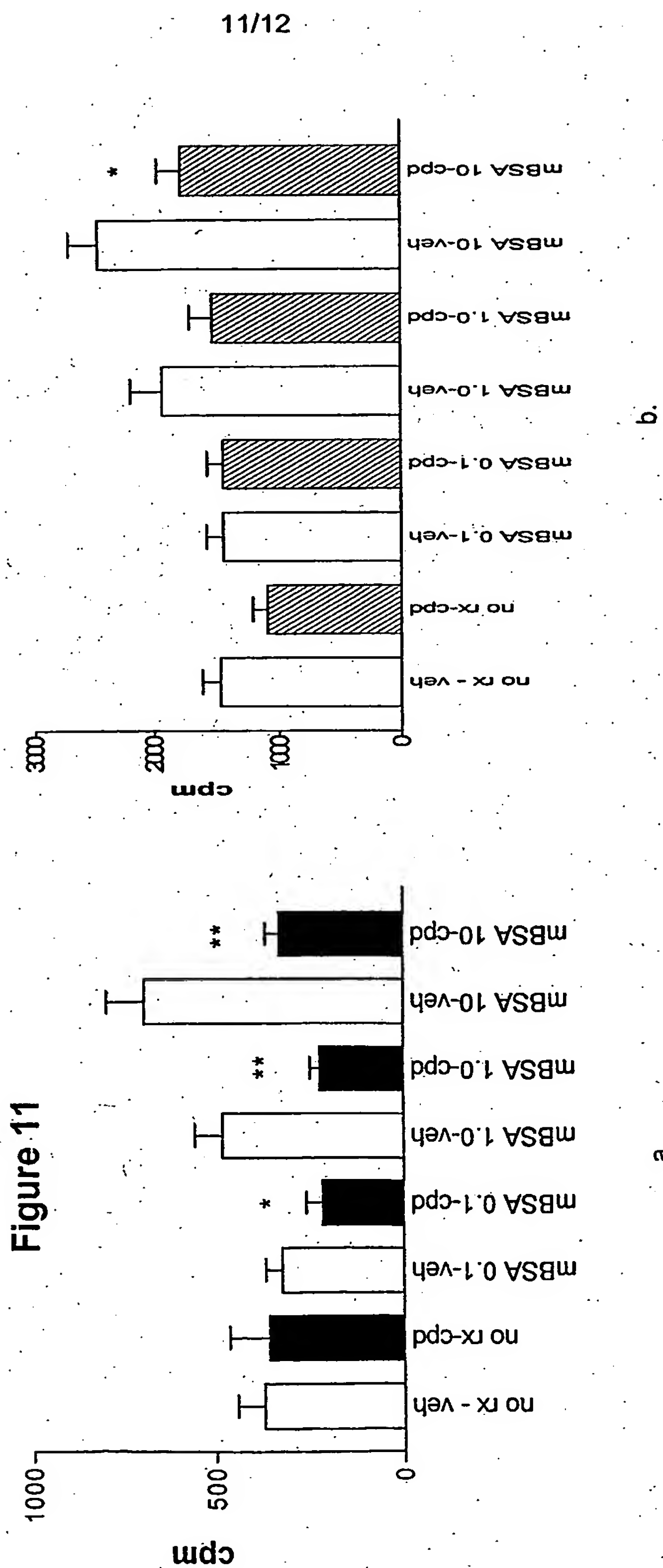
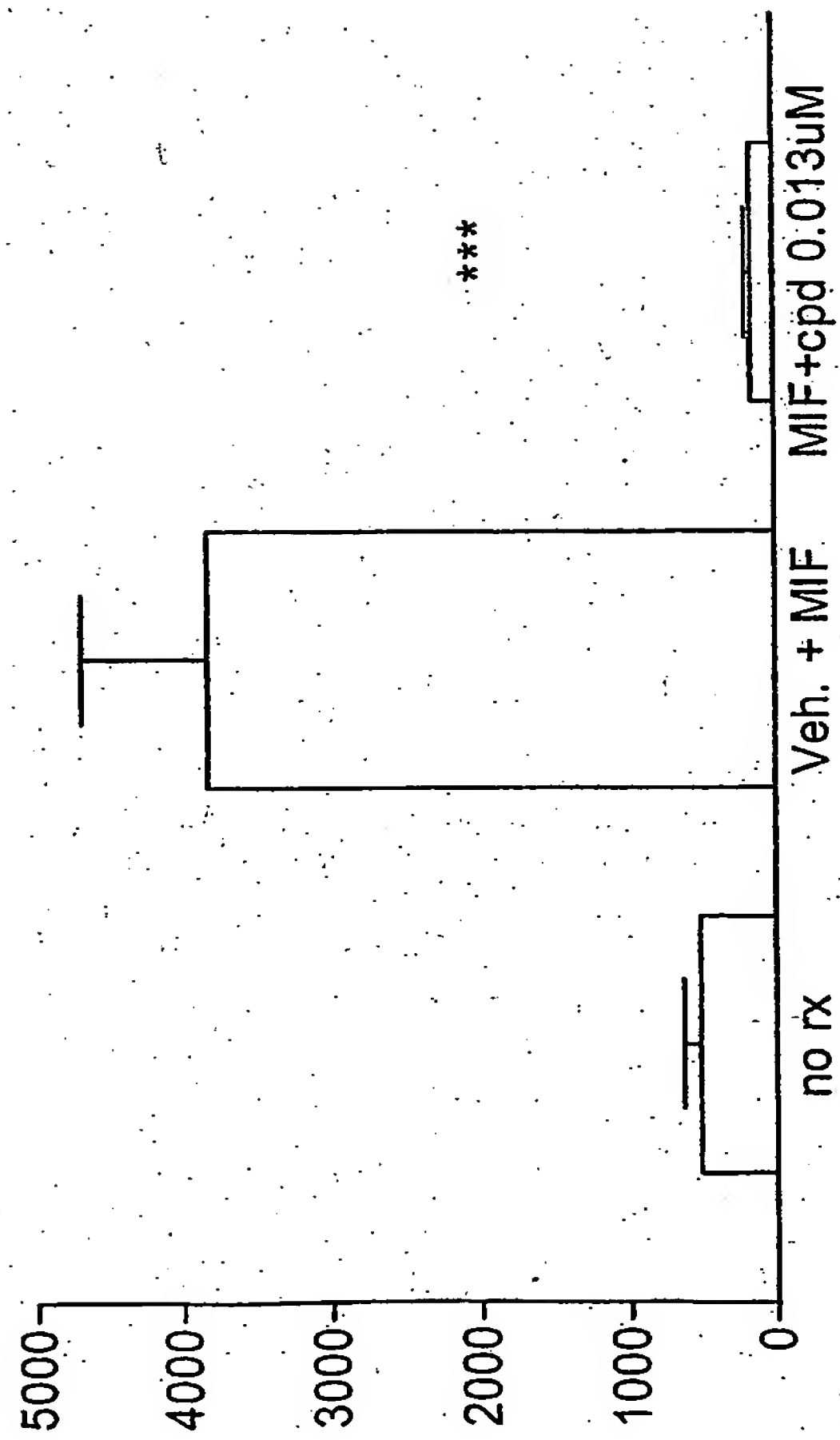


Figure 11

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Figure 12



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU03/00717

A. CLASSIFICATION OF SUBJECT MATTER												
Int. Cl. ⁷ : C07D 235/26, 235/28, 307/83; A61K 31/4184, 31/585												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols)												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPIDS, CA, Medline. Substructure search and keyword search ('MIF', 'macrophage', 'inhibit?', 'factor', '?benzim?', '?benzothi?', '?benzox?', '?benzofur?', 'cytokine')												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
X	WO 01/77083 A1 (GLAXO GROUP LIMITED) 18 October 2001 See page 24 Example 1, and page 6 compound (XII)	31-34										
X	WO 01/92239 A1 (WARNER-LAMBERT COMPANY) 6 December 2001 See examples	31-34										
X	WO 01/44223 A1 (ABBOTT LABORATORIES) 21 June 2001 See examples, claims	31-34										
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex												
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 3 July 2003		Date of mailing of the international search report 18 JUL 2003										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No: (02) 6285 3929		Authorized officer DAVID GRIFFITHS Telephone No : (02) 6283 2628										

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00717

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/36402 A1 (CIBA SPECIALITY CHEMICALS HOLDING INC.) 22 July 1999 See page 12, compound IV, page 13 compound IX, and fourth compound on page 13.	31-34, 36
X	WO 96/10026 A1 (JANSSEN PHARMACEUTICA N.V.) 4 April 1996 See page 6, compound (I-d)	31-34
X	EP 0462831 A2 (IMPERIAL CHEMICAL INDUSTRIES PLC) 27 December 1991 See page 12, line 38; page 13 lines 35 and 51	31-34
X	FR 2688504 A1 (SYNTHELABO SOCIETE ANONYME) 17 September 1993 See compounds of formula (I) and (IV)	31-34
X	US 4400319 (STEFAN HARI ET AL.) 23 August 1983 See columns 6 and 7, Table 1	31-34, 36
X	DD 292452 A5 (KARL MARX UNIVERSITAT) 1 August 1991 See compounds of formula (I) and examples	31-34
X	US 4329477 (RUDIGER SCHUBART ET AL.) 11 May 1982 See whole document	31-34
X	US 3775333 (F. F. LOFFELMAN ET AL.) 27 November 1973 See Example 1	31, 33, 34
X	GB 876015 (CHEMISCHE WERKE ALBERT) 30 August 1961 See first page, right-hand column, lines 69-74	31-34
X	US 2933503 (R. L. CLARK ET AL.) 19 April 1960 See examples	31-34
X	US 2843597 (R. L. CLARK ET AL.) 15 July 1958 See whole document	31-34
X	STN File HCA, Abstract 115:256067, & E. KAWASHIMA ET AL. "2,5,6-Trichlorobenzimidazole. Synthesis of a Precursor for 2-Substituted 5,6-Dichlorobenzimidazoles" Nucleic Acid Chem. 1991, 4, 24-26 Abstract and CAS Registration No. 2033-29-6	31-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/00717

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN File HCA, Abstract 52:77210 & R. L. CLARK ET AL. "Synthesis of Some Substituted Benzimidazolinones" Journal of the American Chemical Society 1958, 80, 1657-1662 Abstract	31-34
X	STN File HCA, Abstract 52:72309 & L. S. EFROS ET AL. "Imidazole Derivatives. XVIII. Nitration of 5-Methylbenzimidazoles" Zhurnal Obshchei Khimii 1958, 28, 62-69 See abstract	31-34

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU03/00717

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos : 1-35 (in part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 1-35 relate to such a broad number and variety of benzo-fused heterocyclic compounds that it was impossible to conduct a search over the entire breadth of the claims. The search has been limited to the benzimidazolin-2-ones disclosed in the examples.
3. ☐ Claims Nos :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU03/00717

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member				
WO	200177083	AU	200156272	EP	1272473		
WO	200192239	AU	200159618	BR	200111301	CA	2409006
		EP	1286975	NO	20025762		
WO	200144223	AU	200122717	BR	200012502	CA	2393027
		EP	1250332				
WO	9936402	AU	26156/99	EP	1047672	US	6087480
WO	9610026	AU	36080/95	BR	9509035	CA	2200579
		CN	1160401	CZ	9700919	EP	784619
		FI	971273	HU	77311	IL	115412
		NO	971375	NZ	293604	PL	319998
		US	5872131	ZA	9508086		
EP	462831	JP	4243865	US	5179115	US	5364877
		US	5462953	US	5278177		
FR	2688504	NONE					
US	4400319	CA	1180325	EP	51560	JP	57094045
DD	292452	NONE					
US	4329477	DE	3008159	EP	35185	JP	56135478
US	3775333	AR	196761	AU	54284/73	BE	801679
		CA	1001804	DE	2332428	FR	2190911
		GB	1379530	IT	985625	JP	49052783
		NL	7306037	ZA	7302152		
GB	876015	NONE					
US	2933503	NONE					
US	2843597	NONE					
END OF ANNEX							